ROLE OF MICROVESSEL DENSITY, HER-2/ NEU EXPRESSION AND CD-34 IN PROGNOSTICATION AND GRADING OF BLADDER CARCINOMAS

Dissertation submitted in partial

fulfilment of the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH - III

GOSCHEN INSTITUTE OF PATHOLOGY AND ELECTRON MICROSCOPY

MADRAS MEDICAL COLLEGE

CHENNAI – 600 003

THE TAMIL NADU
DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI

APRIL 2013

CERTIFICATE

This is to certify that this Dissertation entitled **"ROLE OF MICROVESSEL DENSITY, HER-2/ NEU EXPRESSION AND CD-34 IN PROGNOSTICATION AND GRADING OF BLADDER CARCINOMAS"** is the bonafide original work of **Dr.SURYALAKSHMI. S**, in partial fulfillment of the requirement for M.D., (Branch - III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in April 2013.

Prof. Dr. SUDHA VENKATESH, M.D.,
Professor of Pathology,
Institute of Pathology and EM,
Madras Medical College,
Chennai – 600003.

Prof. Dr. P. KARKUZHALI, M.D.,
Director,
Institute of Pathology and EM,
Madras Medical College,
Chennai – 600003.

Prof. Dr. KANAGASABAI, M.D.,
DEAN**,**
Madras Medical College and Government General Hospital,
Chennai - 600003

DECLARATION

I, **Dr. Suryalakshmi. S,** solemnly declare that the dissertation titled "**ROLE OF MICROVESSEL DENSITY, HER-2/ NEU EXPRESSION AND CD-34 IN PROGNOSTICATION AND GRADING OF BLADDER CARCINOMAS**" is the bonafide work done by me at Institute of Pathology, Madras Medical College under the expert guidance and supervision of **Prof. Dr. Sudha Venkatesh, M.D.,** Professor of Pathology, Institute of Pathology and Electron Microscopy, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr. M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

Date: Dr. Suryalakshmi. S

ACKNOWLEDGEMENT

I take the opportunity to thank **Prof. Dr. KANAGASABAI, M.D.**, Dean, Madras Medical College and Government General Hospital, for the permission given to utilize the vast resources of the Institution.

I also express my sincere thanks to **Prof.Dr.P.KARKUZHALI, M.D.**, Director of Institute of Pathology and Electron Microscopy, Madras Medical College, Chennai for her valuable opinions and encouragement throughout the study.

I express my heartfelt thanks to **Prof.Dr.SUDHA VENKATESH, M.D.**, Professor of Pathology, Institute of Pathology and Electron Microscopy, Madras Medical College for her valuable advice, encouragement and suggestions during the study.

I thank all the Professors, **Prof.Dr.SHANTHA RAVISHANKAR, M.D.**, Neuropathology, **Prof.Dr.GEETHA DEVADAS, M.D., D.C.P.**, **Prof.Dr.M.P.KANCHANA, M.D.**, Institute of Obstetrics & Gynaecology, **Prof.Dr.K.RAMA, M.D.**, Government Kasturba Gandhi Hospital, **Prof.Dr.INDIRA, M.D.**, Regional Institute of Ophthalmology, **Prof.Dr.T.CHITRA, M.D.**, Institute of Child Health and **Prof.Dr.S.PAPPATHI, M.D., D.C.H.** who provided constant support and valuable knowledge during the past 3 years.

I express my thanks to all my Assistant Professors for their help and encouragement during the past 3 years.

I also extend my thankfulness to all my friends, colleagues, technicians of the Institute of Pathology for their help and support for this dissertation.

I express my heartfelt sincere thanks to all my Assistant Professors for their help and suggestions during the study.

I also express my thanks to my family for their constant support during all my efforts.

ABBREVIATIONS

Her-2 neu	-	Human Epidermal Growth Factor Receptor 2
TCC	-	Transitional cell carcinoma
SCC	-	Squamous cell carcinoma
MVD	-	Microvessel density
PUNLMP	-	Papillary urothelial neoplasm of low malignant potential
SD	-	Standard deviation
N	-	Number of cases
IHC	-	Immunohistochemistry
WHO	-	World Health Organisation
Rt lateral wall	-	Right lateral wall
Lt lateral wall	-	Left lateral wall

CONTENTS

INTRODUCTION

Bladder cancer is the sixth common cancer in developed countries[1] and comprises 3.2% of all cancers. Though the mortality due to bladder cancer shows a steady decrease, there is an increase in its incidence, especially in females. It is more common in westernised countries like North America, United kingdom and Australia. The incidence of bladder cancer in India is 3.2% in males and 0.7% in females among all malignancies. The two main causes of bladder cancer have been smoking and industrial exposure. The industrialised countries show a heavy burden of bladder cancer due to increase in smoking, especially in females.[3]

Transitional cell carcinoma (TCC) is the commonest carcinoma in urinary bladder. However, in countries like Egypt and in parts of African continent, squamous cell carcinoma (SCC) predominates.[2] This is due to the endemic presence of schistosomiasis which causes chronic irritation of the bladder.

Though there are many prognostic factors in bladder carcinoma, histological stage and grade of the tumour have been considered as important factors. In some cases, tumours with higher grade and stage behave in an indolent way whereas tumours with lower grade and stage

show high incidence of recurrence. Hence additional prognostic information is required to guide clinicians in the management of these patients.

Microvessel density (MVD) has been considered as an important prognostic factor in many solid tumours and also in carcinomas of bladder.[4]. Immunohistological methods can be used to identify the density of newly formed blood vessels. The hot-spot method is commonly employed where the areas of high MVD are identified in low power and then they are counted in high power. Though there are many other methodologies in assessing microvessel density, no standard technique has yet been adopted either in urology or in general medicine.[4] Increased MVD has been found to be associated with increased stage, grade and increased chances of recurrence.

The HER-2/neu gene is located on chromosome 17 which encodes a 185 kilodalton transmembrane protein. The role of HER-2/neu over-expression has been reported in many tumours like breast, lung, gastric, ovarian and oral cancers. HER-2/neu expression in bladder carcinoma has been studied by many authors and its expression has been found to range from 9% to 81%.[5] This over-expression has been found to be associated with increased grade, stage and poorer outcome. Also, targeted therapy is being tried in cases of HER-2/neu positive tumours.[6]

In this study of 50 cases, an attempt is made to study the role of microvessel density and overexpression of HER-2/neu immuno-histochemically in correlation with stage, grade, recurrence, positive urine cytology and other clinico-pathological parameters.

AIMS AND OBJECTIVES

- To identify the incidence and distribution of bladder carcinoma in patients admitted in Government General Hospital, Chennai during the year 2011-12.
- To study the histo-morphological features of bladder carcinoma including tumour size, tumour location, number, histological type, grade, depth of infiltration, stage, squamous metaplasia and necrosis.
- To study the immunological expression of HER-2/neu in bladder carcinoma.
- To study the immunological expression of CD34 in bladder carcinoma and to find the microvessel density (MVD) by CD 34 expression.
- To evaluate the association of HER-2/neu over expression and MVD in relation to clinical stage, histological grade, urine cytology positivity and with other prognostic factors.
- To assess their prognostic significance by following up the patients for recurrence.

REVIEW OF LITERATURE

Bladder carcinomas are neoplasms of the bladder arising from the lining transitional epithelium. More than 90% of bladder cancer cases are urothelial (transitional cell) carcinomas which originate in the epithelial cells that line the bladder wall internally.[7] About 5% of bladder carcinomas are squamous cell carcinomas, and less than 2% are adenocarcinomas; small cell carcinoma is less common.[7,9] Bladder carcinomas can be classified into two types-superficial (80%) and invasive disease(20%), on the basis of their histological appearance. The non-muscle-invasive disease has a high recurrence of about 50–70% but have a low progression rate of about 15–25% of the patients.[1,3]

EPIDIMEOLOGY:

Bladder cancer is the sixth most common malignancy in developed countries.[1,3] It ranks as the fourth and ninth most frequently diagnosed cancer in men and women, respectively, in the United States.[1] The most common type of bladder cancer is transitional cell carcinoma, now commonly called the urothelial carcinoma, which is derived from the urothelium, constituting more than 90% of bladder cancer cases. Squamous cell carcinoma accounts for 1.1% and 2.8% of all bladder cancers in men and women respectively. However, its incidence is

increased in countries where schistosomiasis is endemic.[2] Adenocarcinoma of the bladder constitutes 1.5% and 1.9% in men and women respectively, of all bladder tumours worldwide.[15]

ETIOLOGY:

The etiology of bladder cancer is not yet fully understood; it has been classically associated with both exogenous and environmental risk factors. The two well known risk factors for bladder carcinoma are smoking and occupational exposure.[3]

Smokers have 2-4 times the risk of the general population, with heavy smokers being at five times the risk. The exact mechanism by which tobacco causes bladder cancer is not yet known, but urothelial carcinogens such as acrolein, 4-amino-biphenyl, arylamine, and oxygen free radicals have been implicated. The experimental work of McDonald and Lund[10] confirmed the carcinogenic properties of β-naphthylamines in the production of bladder cancer. Importantly, they also demonstrated that the route of exposure to the carcinogen was not the blood stream but the urine.

Occupational exposure to aniline dyes, aromatic amines such as 2-naphthylamine and benzidine has been implicated as the second most common risk factor for bladder cancer. Benzidine, which is the most carcinogenic aromatic amine, has been primarily used in dye production

and as a hardener in the rubber industry. Aromatic amines are now prohibited, but people exposed to chemical substances from the combustion of coal are also known to have an increased risk of bladder carcinoma. Occupational bladder cancer has also been observed in gas workers, painters, and hairdressers.

Nutrition also plays a role in bladder cancer. Vitamin A supplement showed a reduced risk of bladder cancer, while fried food and fat caused an increased risk.[3] A high intake of fluid was shown to reduce the risk of bladder cancer in one study but this remains controversial.[3]Research from Taiwan and Chile has shown an increased risk of bladder cancer in populations using drinking water with a high content of arsenic. Hence, these and other water contaminants are being actively investigated.[3] Consumption of coffee and artificial sweeteners were implicated in bladder Carcinoma.[11,12]

Other factors implicated in developing bladder cancer and its progression include analgesic use, chemotherapeutic agents such as cyclophosphamide,[13,14] pelvic radiation, urinary tract infections including bacterial, parasitic, fungal, and viral infections and stones in bladder. There is a causative relationship between the parasite bilharzias and squamous cell carcinoma of the bladder, frequently seen in the Middle East patients.[3]

HISTOLOGICAL TYPES:

The WHO classification is used for bladder carcinomas (Annexure I). Approximately 90% of bladder neoplasms are urothelial carcinomas. The remaining 10% comprise all other types of carcinoma, a small number of sarcomas, and miscellaneous tumours. The classification of urothelial neoplasms is

Non-invasive urothelial neoplasms[76]:

Urothelial papilloma is a benign neoplasm composed of a delicate fibrovascular core covered by normal looking urothelium. The incidence is <1% of all bladder tumours.[78] It was first introduced in WHO classification 1973 and is defined as the same in 2004 WHO/ISUP classification.

Inverted urothelial papilloma is a benign neoplasm resembling papilloma but having an inverted growth pattern with normal to minimal cytologic atypia of the cells.[79]

Papillary urothelial neoplasm of low malignant potential (PUNLMP) is a non-invasive papillary urothelial tumour resembling the exophytic urothelial papilloma but shows increased layers of epithelium compared to the thickness of normal urothelium.[77] The terminology of

PUNLMP was first introduced to replace the previously designated WHO grade 1 urothelial carcinoma that was defined by the 1998 WHO/ISUP classification system. Murphy[81] in 1999, interpreted some of the 1973 WHO grade 1 tumours as PUNLMP. Later, Cheng et al[84] reported a series of 112 patients in 2007 whose bladder tumours showed findings consistent with PUNLMP, with up to 35 yr of follow-up (median, >12 yr), with tumour recurrence in 29% of patients. Bostwick and Mikuz[82] translated the 1973 WHO grade 1, 2, and 3 tumours as PUNLMP, low grade papillary urothelial carcinoma, and high grade papillary urothelial carcinoma respectively in the year 2002. Reuter and Melamed[83] interpreted the 1973 WHO grade 1 tumours as PUNLMP, grade 2 tumours as low grade papillary urothelial carcinoma, and grade 3 tumours as high grade papillary urothelial carcinoma.

Non-invasive low grade papillary urothelial carcinoma is a neoplasm composed of transitional cells lining papillary fronds showing an orderly appearance, but the variations in architecture and cytologic features are easily recognisable.[75]

Non-invasive high grade papillary urothelial carcinoma is a neoplasm composed of urothelium lining the papillary fronds with moderate to marked architectural and cytologic atypia.

Carcinoma-in-situ is a non-papillary, flat, lesion in which the surface epithelium contains cells that are cytologically malignant.[73,74] Primary carcinoma-in-situ which arises de novo and accounts for 1–3% of urothelial neoplasms is most commonly seen in the bladder. The distal ureters can be involved in 6–60%, the prostatic urethra in 20–67%, and the prostate ducts and acini in up to 40%.[74]

Infiltrating urothelial carcinoma:

These neoplasms are similar to non infiltrating neoplasms except for the key feature of invasion which is characterized by nests, clusters, or the presence of single cells within the papillary cores, lamina propria or into the muscle.

This type has numerous variants[50] which include

Infiltrating urothelial carcinoma **with squamous differentiation**[51] which shows the presence of nests of malignant squamous epithelium, characterized by polygonal cells that frequently display dyskeratosis, keratin pearl formation, and occasional intercellular bridges. Squamous differentiation within urothelial carcinoma occurs in about 21% of urothelial carcinomas of the bladder and is associated with poorer prognosis due to the resistance of squamous elements to chemotherapy and radiotherapy.

Glandular differentiation[51] Glandular differentiation within urothelial carcinoma was first described in the literature in 1968. It occurs in 6% of urothelial carcinomas and is defined by the presence of true glandular spaces within urothelial carcinoma. These glandular structures consist of tubular glands or glands resembling enteric epithelium, often associated with variable mucin production.

Trophoblastic differentiation[52] was described in 1904. Since then, more than 30 cases of tumours with trophoblastic differentiation, including cases of pure choriocarcinoma, have been reported. It encompasses a wide range of patterns, including formation of syncytiotrophoblast, formation of areas resembling pure choriocarcinoma, and urothelial carcinomas without giant cells that express human chorionic gonadotropin. The presence of trophoblastic differentiation is associated with poorer prognosis.

Carcinomas with a **nested pattern**[53] are very rare with an incidence of 0.3%. In 1979, Stern made the initial observation of an unusual benign-looking bladder carcinoma of “Brunn's nest origin,” and since then more than 50 cases have been reported. It is characterized by tumour cells with a nested growth pattern, which can be confused with von Brunn nests, cystitis cystica, and nephrogenic adenoma. These tumours commonly present at high disease stage.[54] Drew et al[54] found

that nested variant tumours are associated with progressive or recurrent disease.

The **microcystic variant**, has micro and macrocysts and tubular structures containing granular eosinophilic material which is PAS and Alcian blue positive and necrotic cellular debris. Atleast 25% of the tumour should be comprised of microcystic component to designate a tumour as microcystic urothelial carcinoma. Microcystic urothelial carcinoma was first described in 1991 by Robert H. Young and Lawrence R. Zukerberg.[55] Till now, 10 cases[56,57,58] have been reported. The presentation and prognosis are similar to conventional invasive urothelial carcinoma.[56,57,58]

The **micropapillary variant** is characterized by a conventional infiltrating urothelial carcinoma with an admixed micropapillary pattern involving the surface urothelium which consist of slender, delicate filiform papillary processes that do not contain distinct fibrovascular cores which on cross section, have a glomeruloid appearance. Micropapillary variant of urothelial carcinoma was first described by Amin and colleagues[59] in the urinary bladder in 1994. At least 115 cases[59,60,561] have been described so far. The micropapillary variant of urothelial carcinoma is associated with a poorer prognosis, such that even

in the absence of muscularis propria in the biopsy, the muscle invasion is considered as present.

Lymphoepithelioma-like variant is defined as a urothelial carcinoma that histologically resembles lymphoepithelioma of the nasopharynx. It is characterized by proliferation of primitive anaplastic cells infiltrating with a prominent, lymphocytic background. Unlike lymphoepithelioma of the nasopharynx, Epstein Barr virus has not been found either using immunohistochemistry or in situ hybridization technology.[62] Pure and predominant forms of lymphoepithelioma-like carcinoma have a more favourable prognosis. When the lymphoepithelioma - like morphology is only focally present, the expected behavior is the same as for conventional urothelial carcinoma of the same grade and stage.

The **lymphoma-like and plasmacytoid variants** of urothelial carcinoma exhibit the morphologic features of lymphoma or plasmacytoma. This variant of urothelial carcinoma was first described in 1991 by Zukerberg et al[63] and Sahin et al.[64] Till now, at least 30 cases of this variant of urothelial carcinoma have been reported.[64,65,66] Of the cases reported, the clinical course following diagnosis has been variable with some patients progressing rapidly to death, whereas others have presented with better survival.

The **giant cell variant** of urothelial carcinoma is characterized by the presence of tumour giant cells exhibiting marked nuclear atypia, along with a component of conventional urothelial carcinoma. These tumours have some similarity to the giant cell tumours of the lung. The significance of diagnosing the giant cell variant of urothelial carcinoma is that it is associated with a poor prognosis.[67,68]

Sarcomatoid variants are a group of biphasic malignant neoplasms exhibiting morphologic evidence of both epithelial and mesenchymal differentiation. More than 100 cases have been reported since the mid 1800s, many originally classified as carcinosarcoma, but in general this is a rare tumour.[69,70] The significance of diagnosing this lesion lies in its association with a poor prognosis. These tumours are typically diagnosed at advanced local stage, and they often exhibit nodal or distant metastases. 70% of patients will die within 2 years of diagnosis.

The **undifferentiated carcinoma**[71] category includes rare tumours that cannot be otherwise classified and usually exhibit high-grade malignant morphology.

Clear cell differentiation has been discussed as a distinct variant of urothelial carcinoma in 1995 since the presentation of 2 cases was done by Kotliar et al.[72] At least 10 cases have been reported, but the true

incidence is unclear because of the recent recognition of this type as a separate entity.

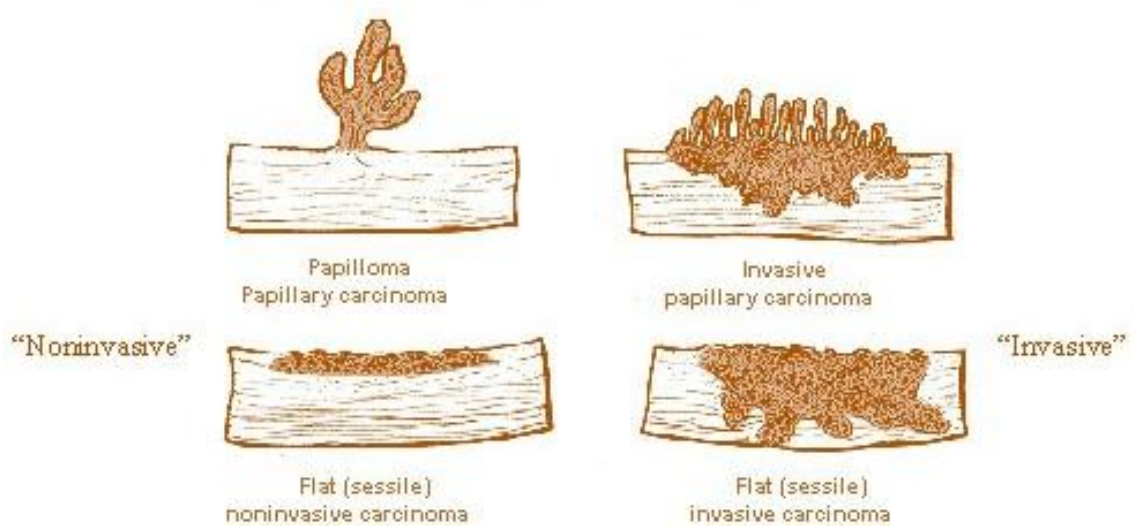


Figure 1: Gross types of bladder cancers

HISTOLOGICAL GRADING:

Histologic grading is one of the most important prognostic factors in bladder cancer. Several attempts at grading were done by many pathologists.

The main such systems were those of Ash in 1940, Mostofi's modification of Ash in 1960 (adopted by the American Bladder Tumour Registry), Bergkvist et al. in 1965, and Malmstrom et al. in 1987. The first widely accepted grading system for papillary urothelial neoplasms was the WHO (1973) classification system, which divided urothelial tumours into four categories: papilloma, grade 1 carcinoma, grade 2, and

grade 3.[16] Histologic grading is based on the degree of cellular anaplasia, with grade 1 tumours having the least degree of anaplasia, but compatible with a diagnosis of malignancy; grade 3 tumours have the most severe degree of anaplasia, and grade 2 have an intermediate degree of cellular anaplasia. Anaplasia is further defined by the authors of the WHO (1973) classification as increased cellularity, nuclear crowding, alteration in polarity of cells, failure of differentiation from the base to the surface, nuclear pleomorphism, variations in nuclear chromatin pattern, displaced, abnormal mitotic figures, and giant cells. The WHO grading system divides bladder cancer into 4 types based on grade such as papilloma, grade1TCC, grade 2 TCC and grade 3 TCC.

PAPILLOMA:

This is a benign neoplasm having papillae lined by normal appearing transitional epithelium.

GRADE 1 UROTHELIAL CARCINOMA:

Grade 1 papillary carcinoma consists of an orderly arrangement of transitional cells lining delicate papillae with minimal architectural abnormality and minimal nuclear atypia. The urothelium is often thickened to more than seven cell layers but there is minimal complexity and fusion of the papillae. The urothelium displays normal maturation

and cohesiveness, with intact superficial layer of cells. They have fine granular chromatin and mitotic figures are usually rare. They are commonly seen around the ureter (69%). Since recurrence can still occur in these patients, long term follow-up is recommended for these cases.

GRADE 2 UROTHELIAL CARCINOMA:

Grade 2 carcinomas retain some of the orderly architectural appearance and maturation of grade 1 carcinoma, but they display focal moderate variation in orderliness. Cytologic abnormalities are present, with moderate degree of nuclear crowding, moderate loss of cell polarity, moderate degree of nuclear hyperchromasia, moderate anisonucleosis, and occasional prominent nucleoli. Mitotic figures can be seen but are usually limited to the lower half of the urothelium. Superficial cells (umbrella cells) are usually present.

Some authors consider both nuclear pleomorphism and mitotic count as criteria for subdividing grade 2 urothelial cancer (grade 2A and 2B), and they have been successful in identifying groups of patients with urothelial cancers with different outcomes.[17,18] However, sub classification of grade 2 urothelial carcinoma is not recommended by some authors because of significant inter observer variability. The risk for

recurrence in patients of grade 2 urothelial carinoma is 45–67% and progression to invasion can occur in about 20% of patients.

GRADE 3 UROTHELIAL CARCINOMA:

There is obvious loss of normal architecture and cell polarity, and frequent atypical mitotic figures. The superficial cell layer is partially or completely absent, accompanied by prominent cellular dyscohesion. Cellular anaplasia characterised by hyperchromasia, nuclear crowding, failure of differentiation and giant cells is frankly evident. Mitosis can be seen in all levels of epithelium and multiple nucleoli may be evident. The papillae may appear fused and branching. The recurrence risk for patients with non-invasive grade 3 cancer is 65–85%, with in cases of invasive cancer recurrence occurs in 20–52% of patients.

In 1998, a revised system for grading non-invasive papillary urothelial neoplasms of the urinary bladder was proposed and was subsequently formally adopted by the World Health Organization.

In 2004, a grading system for non-invasive papillary urothelial neoplasms was given by International Society of Urologic Pathology (ISUP), which was later accepted by WHO. According to this new system, noninvasive papillary urothelial neoplasms are divided into four categories namely papilloma, papillary urothelial neoplasm of low

malignant potential (PUNLMP), low-grade papillary urothelial carcinoma, and high-grade papillary urothelial carcinoma.

INTERNATIONAL SOCIETY OF UROLOGICAL PATHOLOGY/ WHO(2004) CLASSIFICATION:

PAPILLOMA:

This entity is similar to the one described in 1973 WHO classification consisting of papillae lined by benign looking transitional epithelium.

PAPILLARY UROTHELIAL NEOPLASM OF LOW MALIGNANT POTENTIAL (PUNLMP):

PUNLMP is a low-grade urothelial tumour with a papillary architecture. This lesion is histologically defined by the WHO (2004) classification system as a papillary urothelial tumour resembling the exophytic urothelial papilloma, but with increased cellular proliferation exceeding the thickness of normal urothelium and minimal atypia; polarity is generally preserved in these tumours. All such tumours would have been considered grade 1 urothelial carcinomas by the WHO 1973 grading system.

LOW GRADE UROTHELIAL CARCINOMA:

A low-grade papillary urothelial carcinoma shows slender papillae with frequent branching and variation in nuclear polarity, nuclei show enlargement and irregularity; chromatin is vesicular, and nucleoli are often present. Mitotic figures may occur at any level. The majority of these cases would have been considered as grade 2 in the WHO (1973) classification. Most patients have a single tumour in the posterior or lateral bladder wall. However, 22% of patients with low-grade papillary urothelial carcinoma have two or more tumours. Tumour recurrence, stage progression and tumour-related mortality are 50%, 10% and 5%, respectively.

HIGH GRADE UROTHELIAL CARCINOMA:

The cells lining the papillary fronds show an obviously disordered arrangement with cytologic atypia. All tumours classified as grade 3 in the 1973 WHO scheme, as well as some assigned grade 2 in that classification, would be considered high grade carcinoma in the 2004 WHO classification. The papillae are frequently fused. The nuclei are pleomorphic with prominent nucleoli. Polarity is altered. Mitotic figures are frequent. Carcinoma in situ is frequently evident in the adjacent mucosa. These tumours can occur as single or multiple lesions. Stage

progression and death due to disease can be seen in as many as 65% of patients.

Other recent proposals for bladder cancer grading:

The Ancona 2001 refinement of the 1973 WHO classification[19] divides urothelial tumours into two main groups based on growth pattern: flat and papillary. Flat tumours include reactive changes, dysplasia, and carcinoma in situ. Papillary tumours include papilloma, grade 1 papillary carcinoma, grade 2 papillary carcinoma, and grade 3 papillary carcinoma.

The publication of the 1999 WHO blue book introduced a new grading scheme.[20] This new classification retained the three-tiered numbering system (grade 1, grade 2, and grade 3 carcinoma), but tumours formerly classified as 1973 WHO grade 1 were subdivided into PUNLMP and grade 1 tumours. Papillary tumours were subclassified as papilloma, papillary urothelial neoplasm of low malignant potential, grade 1, grade 2, and grade 3 papillary urothelial carcinoma

STAGING:

The TNM staging system (Annexure II) is widely used in western countries. It is the best available predictor of prognosis and is recommended in India also.

Early/Superficial bladder cancer is otherwise called as non-muscle invasive cancer carcinoma in situ, Ta tumours and T1 tumours are included under superficial bladder carcinoma.

Invasive bladder cancer includes T2 and T3 tumours. In T2 lesion the tumour has spread to the muscle layer whereas in case of T3 tumours, it has it has grown through the muscle layer.

Advanced bladder cancer is the one which is widespread outside the bladder. It includes T4 bladder tumours.

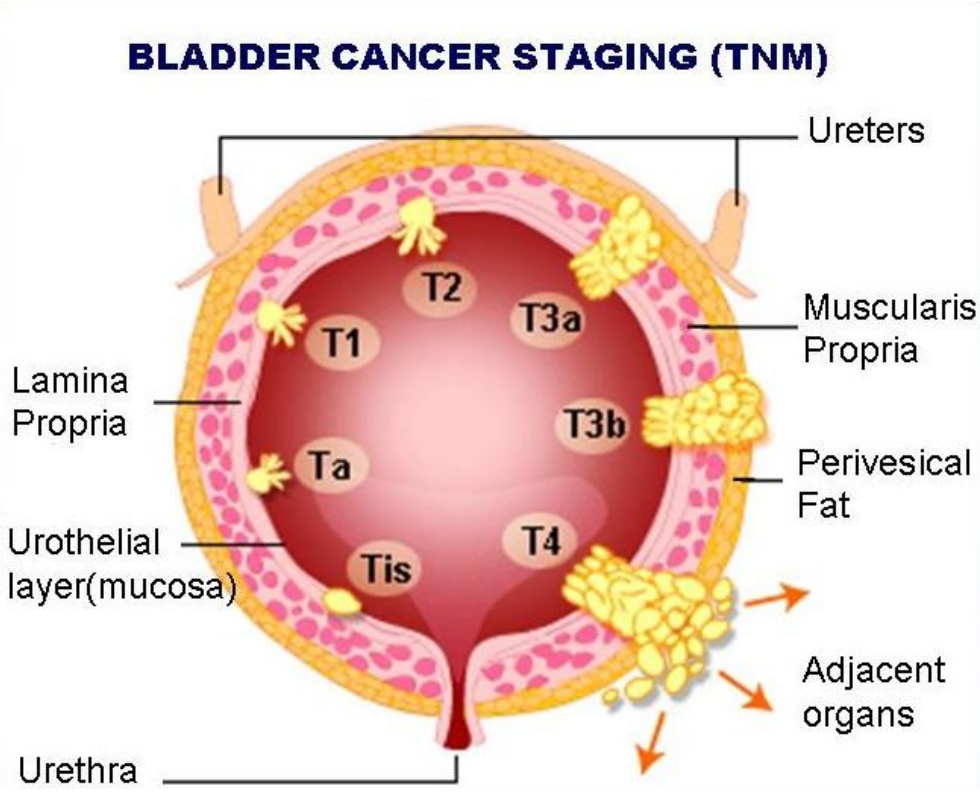


Figure 2: Staging of bladder cancers

PROGNOSTIC FACTORS IN BLADDER CARCINOMA:

Prognostic factor is defined as any variable that provides information useful in assessing the outcome at the time of diagnosis of the disease. The prognostic factors are classified as clinical factors, pathological factors and genetic/molecular factors.

The clinical factors[80] include patient age and a previous history of bladder cancer.

AGE: Younger patients with bladder cancer appear to have a favourable prognosis, because they usually present as superficial tumours and low-grade tumours.

RECURRENCE: Mullerad et al and Kang et al reported that a previous history of bladder cancer was an independent predictor of cancer-specific survival.

The pathological factors play more useful role in assessing prognosis. It includes the following

1. SIZE: Tumour size > 5 cm has been associated with 35% of invasion compared to 9% invasion in small bladder tumours and is associated with poorer prognosis.[21]

2. LOCATION: Tumours arising from the bladder neck are associated with a poorer prognosis. Tumours of the bladder dome tend to present as higher-grade lesions, whereas tumours of the lateral walls and the ureteric orifices tend to be of lower grade.[26]

3. NUMBER: Multiple tumours present more frequently with recurrence (40-90%) when compared to single tumours (18-60%). [22]

3. HISTOLOGICAL TYPE: Non-papillary TCCs tend to present as higher grade, stage and more aggressive tumours.[107]

4. LYMPH NODE INVOLVEMENT: Lymph node involvement is associated with increased chances of recurrence and disease progression and has an overall poor outcome.[108]

5. STAGE: Since Ta lesions are confined to basement membrane they are associated with good prognosis. As the tumour invades the muscle layer the prognosis become poorer.[23]

6. GRADE: Grade is an important prognostic indicator for progression, mortality and recurrence.[24] High grade tumours are associated with increased chances of recurrence and have a high risk of progression to muscle invasive disease.

7. CARCINOMA-IN-SITU CHANGES: Recurrence rates are higher in transitional cell carcinomas which have associated carcinoma-in-situ changes involving the adjacent mucosa.[25]

8. LYMPHO-VASCULAR INVASION: This feature, as determined microscopically with H&E stain or by vascular stains in either lymph vessels or blood vessels, is associated with an increased rate of recurrence.[27]

9. SQUAMOUS METAPLASIA: Squamous metaplasia in transitional cell carcinomas are fairly common and this squamous epithelium resist radiotherapy. Hence they have a poor prognosis when they present in inoperable stage.[90]

The molecular factors which are associated with prognosis are

1. MICROVESSEL DENSITY: This feature is alleged to be an independent prognostic indicator which is associated with increased stage, and more chances of recurrence.[28]

2. P53 OVEREXPRESSION: Nuclear over expression of P53 is related to both grade and stage of bladder carcinoma.[29].

3. ALTERED EXPRESSION OF RB GENE: Tumours exhibiting reduced expression of the RB protein have an aggressive behaviour than those without reduced expression.[30]

4. LOSS OF E-CADHERIN: Tumours showing loss of E-cadherin has worse prognosis than those in which this surface antigen was present.[31]

5. HER 2/NEU EXPRESSION: Increased HER-2/neu expression of this marker is associated with higher grade, stage and metastatic growth.[32]

IMMUNOHISTOCHEMISTRY (IHC):

Albert Coons et al in 1941 first labelled antibodies directly with fluorescent isocyanate. Nakane and Pierce et al in 1966, introduced the indirect labelling technique in which the unlabelled antibody is followed by second antibody or substrate. Various stages of development of immunohistochemistry include peroxidase – antiperoxidase method (1970), alkaline phosphatase labelling (1971), avidin biotin method (1977) and two layer dextrin polymer technique (1993).[33]

Antigen retrieval:

Antigen retrieval can be done by the following different techniques to unmask the antigenic determinants of fixed tissue sections.

1. Proteolytic enzyme digestion
2. Microwave antigen retrieval

3. Pressure cooker antigen retrieval
4. Microwave and trypsin antigen retrieval

PROTEOLYTIC ENZYME DIGESTION:

Huank et al in 1976 introduced this technique to breakdown formalin cross linkages and to unmask the antigen determinants. The most commonly used enzymes include trypsin and proteinase. The disadvantages include over digestion, under digestion and antigen destruction.

MICROWAVE ANTIGEN RETRIEVAL:

This is a new technique most commonly used in current practice. Microwave oven heating involves boiling formalin fixed paraffin sections in various buffers for rapid and uniform heating.

PRESSURE COOKER ANTIGEN RETRIEVAL:

Miller et al in 1995 compared and proved that pressure cooking method has fewer inconsistencies, less time consuming and can be used to retrieve large number of slides than in microwave method.

PITFALLS OF HEAT PRETREATMENT:

Drying of sections at any stage after heat pretreatment destroys antigenicity. Nuclear details are damaged in poorly fixed tissues. Fibers

and fatty tissues tend to detach from slides while heating. Not all antigens are retrieved by heat pretreatment and also some antigens like PGP 9.5 show altered staining pattern.

DETECTION SYSTEMS:

After addition of specific antibodies to the antigens, next step is to visualize the antigen antibody reaction complex. The methods employed are direct and indirect methods.

In the direct method, primary antibody is directly conjugated with the label. Most commonly used labels are flouro-chrome, horse radish peroxidase and alkaline phosphatase. Indirect method is a two-step method in which labeled secondary antibody reacts with primary antibody bound to specific antigen. The use of peroxidase enzyme complex or avidin biotin complex further increases the sensitivity of immunohistochemical stains[33].

In 1993, Pluzek et al introduced enhanced polymer one step staining, in which large numbers of primary antibody and peroxidase enzymes are attached to dextran polymer back bone. This is a rapid and sensitive method[37].

Dextran polymer conjugate two step visualization system is based on dextran technology in Epos system. This method has greater sensitivity and is less time consuming.

Uses of IHC in bladder pathology [34]

1. Assessment of prognosis by using markers for microvessel density (CD 34, CD 31, VEGF), HER-2/neu, P53, EGFR and other markers.
2. Assessment of metastatic lesions of possible bladder origin by using antibodies to CK7 and CK20, uroplakin, thrombomodulin as well as 34βE12 and 4A4.
3. Evaluation of spindle cell lesions to distinguish sarcomatoid carcinoma from mesenchymal lesions.

MICROVESSEL DENSITY:

Tumour stage and histopathological grade are the most important prognostic factors affecting the survival of bladder carcinoma, but tumours with similar stage and grade may show different outcome. Thus there is some evidence that some other prognostic factors also play a role in prognosis of patients. A majority of studies have assessed the prognostic value of measuring tumour angiogenesis (i.e., measurement of tumour microvessel densities) and have found a positive association between increasing microvessel densities and prognosis.[28] Angiogenesis or neovascularization is by definition formation of new capillaries from

pre-existing blood vessels. The process of neovascularization in tumours is regulated by the combined action of tumour cells, stromal cells and inflammatory cells.[38] Neovascularisation is essential for both benign and malignant tumours especially when it grows beyond 2 mm^3. Microvessel density has been shown to add to prognostic information in a number of solid tumours including prostate (Weidner et al, 1993), colon (Takahashi et al, 1995), lung (Mattern et al, 1996) and breast cancer (Linderholm et al, 1999). Microvessel density, has also recently been proven to add prognostic information in bladder carcinoma. However, contradictory results have also been reported;[39] this may be due to significant differences in the methods employed for sample selection, techniques of immunostaining, counting of vessels and statistical analysis, although a number of biological differences may account for the discrepancy.

MICROVESSEL DENSITY BY IMMUNOHISTOCHEMISTRY:

Immunohistochemically, the blood vessels are identified by highlighting them using specific markers. Targeted therapy has been useful in cases of marker positive tumours. The markers can be divided into panendothelial markers and markers which bind selectively with the activated endothelium. The examples of panendothelial markers are CD 31 and CD34 which bind with all small and large vessels. The problems in these markers are staining of other cells- like staining of lymphatics by

CD 31 and staining of inflammatory cells by CD 34. These can be reduced to some extent by pre treatment with microwave oven. The activated endothelial markers like CD 105 bind specifically to the proliferating endothelium and hence do not stain for normal blood vessels or lymphatics. Thus they are more specific as a marker of angiogenesis.

Vascular hot spots are defined as regions of high vascular density within the tumour. This was first described by Weidner et al, 1991in breast cancer.[85] These hot spots can be identified by inspecting the sections under low power. Atleast 10 of these hot spots should be analysed so that the chance of missing them is reduced. They are seen predominantly at the peripheral margins of the tumour. Once the vascular hot spot is identified, they are viewed under high power to count individual microvessels. The counting can be done either manually or by using Computerised Image Analysis systems. Magnifications of the order of 200 – 400X and field sizes ranging from 0.12 to 1.00 mm^2 have been used by Vermeulen et al, (1996). A higher magnification improves the detail of the image and allows the identification of even single endothelial cell. According to Weidner et al (1991), any highlighted endothelial cell or cell cluster clearly separate from adjacent microvessels, tumour cells and other connective tissue elements should be regarded as a distinct countable microvessel. A lumen or the presence of red blood cells is not

necessary to identify a microvessel. However the cut-off calibre size necessary to designate a microvessel has not been specified by many authors and hence even single endothelial sprout and a large calibre vessel are included in counting. Atleast three different areas of vascular hot spots are counted in 200X magnification and the highest of these is taken as the microvessel density.

The mean microvessel density can be calculated after the tumour is divided into characteristic groups. The values are compared and statistical significance is calculated.

Computerised Image Analysis Systems is an automated counting technique that improves reproducibility and reduces inter-observer variability. According to Wakui et al (1992) and Visscher et al (1993), it has been considered as a more objective method of assessing microvessel density. Additional parameters like the number of vessels with a certain dimension range, the vessel luminal area, vessel luminal perimeter and the number of immunostained areas per microscopic field can also be assessed.[28]

HER-2/NEU RECEPTORS:

The HER-2/neu gene was originally called ‘neu’ as it was first derived from rat neuro/glioblastoma cell lines. Coussens and coworkers named it HER2 because its primary sequence was very similar to Human Epidermal Growth Factor receptor (EGFR or ERBB or ERBB1).[86] This human proto-oncogene, also known as c-erbB2, ErbB-2 is a 185-kilodalton transmembrane receptor tyrosine kinase located at chromosome 17q. These proteins belong to subclass I of the super-family of receptor tyrosine kinases. They are expressed in many tissues of epithelial, mesenchymal, and neuronal origin and are critical for cell proliferation and tissue differentiation. The clinical significance of HER-2/neu has already been evaluated in colorectal, breast, stomach, lung, head and neck, pancreatic, urothelial carcinoma, and gliomas and prostate cancers; patients with elevated HER-2/neu demonstrate poor survival compared with patients with lower level of HER-2/neu.[46,47,48]

Several studies have reported an association between prognosis and HER-2/neu gene over expression in bladder cancers. HER 2/neu protein over expression and HER-2/neu gene amplification were correlated with higher histologic tumour grade and invasion in many studies. The first report of increased amplification and over expression of the HER-2/neu in bladder carcinoma was reported by Zhau HE, Zhang X, von

Eschenbach AC, et al in 1990.[40] Till then there were many studies which showed an association of HER-2/neu expression with increased grade and stage in bladder carcinomas.[41,42,43]Some studies also showed that HER-2/neu was an independent variable in determining patient survival.[44,45]T he prevalence of HER-2/neu expression in bladder carcinomas has ranged from 2% to 81% . In a recent study by Lae M, Couturier J, Oudard S, Radvanyi F et al in 2010, HER-2/neu protein over-expression was found in 9.2%, while HER-2/neu gene amplification was found in 5.1% of tumour specimens.[49] HER-2/neu can be assayed by immuno histochemistry for protein over expression and by fluorescent in situ hybridization for gene amplification.

The scoring system which is used for HER-2/neu expression in bladder cancers is

Staining pattern	**Score**	**Her2neu expression**
No staining	0	Negative
Weak staining in part of membrane of less than 10% of the cells.	1	Negative
Complete membranous staining of weak or moderate intensity in >10% of tumour cells.	2+	Positive
Strong complete membranous staining in more than 10 % of tumour cells creating a fish net pattern.	3+	Positive

Positive staining is considered when the score is 2+ or 3+ and the absence of cytoplasmic staining.

MATERIALS AND METHODS

This study is a combined retrospective and prospective descriptive study of bladder carcinomas conducted in the Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai during the period between January 2010 and December 2011.

A total of 19,898 cases were submitted to the Department of Pathology, Rajiv Gandhi Government General hospital during the period of January 2010 to December 2011 for histopathological examination. Out of that, 143 cases were bladder cases. Among them, 13 specimens were radical cystectomies, 2 specimens were simple cystectomies, 90 specimens were TURBT and 38 specimens were small biopsies. The total number of non- neoplastic and malignant cases of the urinary bladder was 27 and 116 respectively.

Out of the malignant cases, 114 cases were carcinomas, one was a case of inflammatory myofibroblastic tumour and another one was a case of leiomyosarcoma of bladder. Out of the 114 cases, 101 were TCCs, 7 cases were adenocarcinomas and 6 cases were SCCs. The TCC cases numbering 101 were taken up for the study.

Source of data:

The bladder carcinoma cases reported in the Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General hospital from January 2010 to December 2011 which have been sent by the Department of Urology.

Inclusion criteria:

All the cases of transitional cell carcinomas reported in bladder specimens irrespective of the age and sex and the procedure done were included for the study.

Exclusion criteria:

- Non-neoplastic lesions and benign neoplasms of the bladder.
- Carcinomas other than transitional cell carcinomas.
- Cases with inadequate material.

Method of data collection:

Detailed history of the cases regarding age, sex, history, type of procedure, site, size, stage, previous surgery details and urinary cytology were obtained for all the 101 transitional cell carcinomas reported during the period of study from surgical pathology records. Hematoxylin and Eosin stained 4 µ thick sections of the paraffin tissue blocks of specimens were reviewed. The following clinical and pathological parameters were

evaluated: Age, gender, tumour size and tumour location (base, lateral, posterior walls and trigone).

Carcinomas were classified as transitional cell type which includes papillary/non-papillary, invasive/non-invasive and any other special types like lymphoepithelial carcinoma, sarcomatoid carcinoma. Other parameters like squamous metaplasia, necrosis and sarcomatoid component were noted. Regarding the depth of invasion, the carcinomas were classified into 4 groups: T1 (invasion of mucosa and submucosa), T2 (invasion of muscle layer in which T2a is invasion of superficial muscle and T2b is invasion of deep muscle), T3 (invasion of perivesical tissue in which T3a means microscopic involvement and T3b means macroscopic involvement) and T4 (invasion of adjacent organs in which T4a is invasion of prostate, uterus and vagina and T4b is involvement of pelvic and abdominal wall), and according to grade the carcinomas were divided into 3 groups: PUNLUMP, low grade urothelial carcinoma and high grade urothelial carcinoma according to the recommendations of the WHO (2004). Carcinoma staging was done according to TNM classification of bladder carcinomas (Annexure-II). The tumours were further evaluated for the presence of infiltration, necrosis, squamous metaplasia and were graded as present or absent. 50 cases of bladder carcinomas of varying grades were randomly selected from the total cases

and their representative formalin fixed paraffin embedded tissue samples were subjected to immunohistochemistry for a panel of 2 markers- CD 34 and HER-2/neu.

Immunohistochemical evaluation:

Immuohistochemical analysis of markers CD 34 and HER-2/neu were done in paraffin embedded tissue samples using Super-sensitive polymer HRP system based on non-biotin polymeric technology. 4 µ thick sections from formalin fixed paraffin embedded tissue samples were transferred on to gelatin coated slides. Heat induced antigen retrieval was done. The antigen was bound with mouse monoclonal antibody (Biogenex) against CD 34 protein and HER-2/neu protein and then detected by the addition of secondary antibody conjugated with horse radish peroxidase-polymer and diaminobenzidine substrate. The step by step procedure of Immunohistochemistry is given in Annexure III.

Antigen	**Vendor**	**Species(clone)**	**Dilution**	**Positive control**
CD 34	Biogenex	Mouse	Ready to use	Bladder
HER-2/neu	Biogenex	Mouse	Ready to use	Bladder

Interpretation and scoring system:

The immunohistochemically stained slides were analyzed for the presence of reaction, cellular localization, percentage of cells stained and intensity of reaction. Membrane staining was assessed for HER-2/neu and cytoplasmic staining for CD 34.

HER-2/neu immuno-reactivity was assessed as being positive when tumours exhibited intense nuclear staining and absent cytoplasmic staining and was categorized into 2 groups: 2+ (complete membranous staining of weak or moderate intensity in >10% of tumour cells) and 3+(strong complete membranous staining in more than 10 % of tumour cells creating a fish net pattern)

CD 34 staining in the endothelial cells was noted. Any highlighted endothelial cell or cell cluster clearly separate from adjacent microvessels, tumour cells and other connective tissue elements were regarded as a distinct countable microvessel. A lumen or the presence of red blood cells was not taken as an essential criteria to identify a microvessel. Hot spots were identified and atleast 3 such hot spots were identified under 200X power and the vessels were counted. The maximum of these was taken as microvessel density. The mean microvessel density was calculated in relation to the prognostic factors.

Statistical analysis :

The statistical analysis was performed using statistical package for social science software version 11.5 which consisted computing the frequency counts and percentages for qualitative variables and mean for the quantitative variables. The expression of microvessel density was correlated with clinico- pathological factors like gender, tumour site, tumour configuration, size, histological types, histological grade, depth of infiltration, stage, squamous metaplasia, necrosis, sarcomatoid component, urine cytology and recurrence using the Student t-test and Anova t-test. HER-2/neu was also correlated with these parameters using Pearson's Chi –Square test.

OBSERVATION AND RESULTS

In the study period of 24 months from January 2010 to December 2011, a total of 19,898 specimens were received in the Institute of Pathology, Madras Medical College for histological examination. Total numbers of bladder specimens received were 143. The total number of non-neoplastic and malignant cases was 27 and 116 respectively. Out of 116 malignant cases 114 cases were carcinomas, 1 was a case of Inflammatory myofibroblastic tumour of bladder and another 1 was a case of leiomyosarcoma. Thus the distribution of non-neoplastic lesions was 20.9%, and of malignant tumours were 81.1% among the bladder specimens.

Among the 143 bladder specimens, there were 13 radical cystectomies, 2 simple cystectomies, 90 TURBTs and 38 small biopsies. All 13 radical cystectomies were done to treat bladder carcinoma. Out of 2 simple cystectomies 1 was done for carcinoma and 1 for inflammatory myofibroblastic tumour.

Of the 114 total carcinomas arising from the bladder, transitional cell carcinomas were the most common constituting 101 cases accounting for 88.6% of carcinomas. Adenocarcinoma of bladder constituted 7 cases accounting for 6.1% and squamous cell carcinoma constituted 6 cases

accounting for 5.3% of carcinomas arising from urinary bladder (Table 1 and chart 1)

TABLE : 1 - HISTOLOGICAL SUBTYPES OF BLADDER CARCINOMAS

Histological subtypes	Number of cases(N)	Percentage
Transistional cell carcinoma	101	88.6%
Adenocarcinoma	7	6.1%
Squamous cell carcinoma	6	5.3%
Total number of cases	114	100%

Transitional cell carcinomas had a peak incidence in the age group of 61-70 years. The youngest age of presentation of bladder cancer was at 22 years in this study. The mean age was 58.74. (Table 2 and chart 2)

TABLE : 2 - AGE WISE DISTRIBUTION OF TRANSITIONAL CELL CARCINOMAS

Age group	Number of cases	Percentage
21 - 30 years	1	1%
31 - 40 years	7	7%
41 - 50 years	17	16.8%
51 - 60 years	29	28.7%
61 - 70 years	32	31.7%
71 - 80 years	13	12.9%
81 - 90 years	2	1.9%
Total cases	101	100%

Among the 101 cases of transitional cell carcinomas, 79 (78.2%) cases were reported in males and 22 (21.8%) cases were reported in females. (Table 3 & Chart 3)

TABLE : 3 - SEX DISTRIBUTION IN TRANSITIONAL CELL CARCINOMAS

Sex	Total number of cases	Percentage
Male	79	78.2%
Female	22	21.8%
Total	101	100%

In this study, 38(37.7%) cases involved the right lateral wall, 30(29.8%) cases involved the left lateral wall, 4(3.9%) involved the posterior wall, 6(5.9%) involved the base, 7(6.9%) involved the dome, 10(9.9%) involved the entire bladder and in 6(5.9%) cases the tumour was multiple. (Table 4 and Chart 4)

TABLE : 4 - DISTRIBUTION OF SITE OF INVOLVEMENT IN TRANSITIONAL CELL CARCINOMAS

Site of bladder cancer	Number of cases	Percentage
Right lateral wall	38	37.7%
Left lateral wall	30	29.8%
Posterior wall	4	3.9%
Base	6	5.9%
Dome	7	6.9%
Entire bladder	10	9.9%
Multiple	6	5.9%
Total	101	100%

Among the 101 cases, 95 tumours (94%) were single and 6 tumours were multiple (6%) (Table 5 and chart 5).

TABLE : 5 - TUMOUR NUMBER IN TRANSITIONAL CELL CARCINOMAS

Tumour number	Number of cases	Percentage
Single	95	94%
Multiple	6	6%
Total	101	100%

The mean size of the tumours which ranged from 0.5 to 10 cm was 4.1 cm. 65(64.4%) were $\leq$ 4cm and 36(35.6%) were > 4cm (Table 6 and chart 6).

TABLE : 6 - DISTRIBUTION OF SIZE IN TRANSITIONAL CELL CARCINOMAS

Size	Number of cases	Percentage
$\leq$4cm	65	64.4%
>4cm	36	35.6%
Total	101	100%

Among the 101 transitional cell carcinomas, 83 cases were papillary and 18 cases were non-papillary. (Table 7 and chart 7)

TABLE : 7 - HISTOLOGICAL APPEARANCE OF TRANSITIONAL CELL CARCIINOMAS

Appearance	Number of cases	Percentage
Papillary	83	82.2%
Non-papillary	18	17.8%
Total	101	100%

Among the 101 transitional cell carcinomas, 74 cases were low grade and 27 cases were high grade. (Table 8 and chart 8).

TABLE : 8 - DISTRIBUTION OF HISTOLOGICAL GRADE IN TRANSITIONAL CELL CARCINOMAS

Grade	Number of cases	Percentage
Low grade	74	73.3%
High Grade	27	26.7%
Total	101	100%

In this study, 71 cases were infiltrative and 30 cases were non-infiltrative (Table 9).

TABLE : 9 - INFILTRATION IN TRANSITIONAL CELL CARCINOMAS

Infiltration	Number of cases	Percentage
Infiltrative	71	70.3%
Non-infiltrative	30	29.7%
Total	101	100%

Out of the 71 infiltrative cases, 41 cases(57.8%) showed infiltration upto subepithelial connective tissue, 23 cases(32.4%) showed infiltration upto superficial muscle layer, 3 cases(4.2%) showed infiltration upto deep muscle layer, 1 case(1.4%) showed microscopic involvement of perivesical tissue and 3 cases(4.2%) showed involvement of prostrate. There were no cases with lymphnode involvement. (Table 10).

TABLE : 10 - DISTRIBUTION OF TRANSITIONAL CELL CARCINOMAS ACCORDING TO DEPTH OF INVASION

Depth of invasion	Number of cases	Percentage
Ta	30	29.7%
T1	41	40.6%
T2a	23	22.7%
T2b	3	3%
T3b	1	1%
T4a	3	3%
Total	101	100%

In the present study, 41 cases (57.7%) belonged to stage I, 26 cases (36.6%) belonged to stage II and 4 cases (5.7%) belonged to stage III. (Table 11 and Chart 9)

TABLE : 11 - DISTRIBUTION OF TRANSITIONAL CELL CARCINOMAS ACCORDING TO STAGE

STAGE	NUMBER OF CASES	PERCENTAGE
0	30	29.7%
I	41	40.6%
II	26	25.7%
III	4	4%
Total	101	100%

In this study, squamous differentiation was seen in 15 cases, necrosis was seen in 19 cases and sarcomatous component was seen in 3 cases. (Table 12 and chart 10)

TABLE : 12 - DISTRIBUTION OF OTHER PROGNOSTIC FACTORS IN TRANSITIONAL CELL CARCINOMA

Patient characteristics	Present	Absent	Total
Squamous metaplasia	15(14.9%)	86(85.1%)	101(100%)
Necrosis	19(18.8%)	82(81.2%)	101(100%)
Sarcomatous component	3(3%)	97(97%)	101(100%)

Among the 101 cases, urine cytology was done in 24 cases, out of which 14 cases were positive for malignant cells and 10 cases were negative for malignant cells. (Table 13 and chart 11)

TABLE : 13 - URINE CYTOLOGY IN TRANSITIONAL CELL CARCINOMAS

Urine cytology	Number of cases	Percentage
Positive	14	58.3%
Negative	10	41.7%
Total	24	100%

In our study, 30 cases were associated with recurrence. Among these cases, 6 cases showed recurrence in 1 month, 6 cases in 2 months, 3 case in 3 months, 1 case in 4 months, 1 case in 6 months, 1 case in 8 months, 7 cases showed recurrence in 1 year, and 1 case in 2 yrs. 3 of the cases were late recurrences with previous tumour occurring in 3 yrs and 1 case recurring after 7 years. (Table 14 and chart 12).

TABLE : 14 - RECURRENCE IN TRANSITIONAL CELL CARCINOMAS

Recurrence	Number of cases	Percentage
1 month	6	20%
2 month	6	20%
3 month	3	10%
4 month	1	3.3%
6 month	1	3.3%
8 month	1	3.3%
1 year	7	23.4%
2 year	1	3.3%
3 year	3	10%
7 year	1	3.4%
Total	30	100%

RESULTS OF IMMUNOHISTOCHEMICAL STUDIES

Of the total 101 transitional cell carcinomas, 50 cases of varying grade and stage were selected in a random manner and subjected to immunohistochemical analysis with a panel of 2 markers – CD 34 and HER-2/neu. The microvessel density was found out by using CD 34.

Of the 50 cases, there were 42 males (84%) and 8 females (16%). The ages ranged between 35 and 81 yrs with a mean age of 60.28. There were 3 cases (6%) of small biopsy, 44 cases of TURBT (88%), 2 cases (4%) of simple cystectomy and 1 case (2%) of radical cystectomy. There were 37 cases (74%) below 66 years of age and 13 cases (26%) more than 66 years. The tumour was located in the right lateral wall in 16 cases, left lateral wall in 13 cases, base in 4 cases, dome in 5 cases, posterior wall in 1 case, entire bladder in 6 cases and the tumour is multiple in 5 cases. The tumours ranged in size from 0.5 to 8 cm with a mean size of 4.32.

Among the final study group, 30 cases (60%) were low grade and 20 cases (40%) were high grade. 45 cases (90%) showed papillary morphology and 5 cases (10%) showed flat morphology. Squamous metaplasia was seen in 9 cases (18%) and necrosis was seen in 8 cases (16%). Sarcomatoid area was seen in 1 case (2%). 8 cases (16%) belonged to stage Ta (non-invasive), 23 cases (46%) belonged to T1, 16 cases (32%) belonged to T2a, 1 case (2%) belonged to T2b, 1 case (2%)

belonged to T3b and 1 case (2%) belonged to T4a. 8 cases (16%) belonged to stage 0, 23 cases (46%) belonged to stage 1, 17 cases (34%) belonged to stage 2 and 2 cases (4%) belonged to stage 3.

Urine cytology was done in 12 cases and was found to be positive in 8 cases (66.7%) and negative in 4 cases (33.3%).Among the 50 cases, 19 cases (38%) showed evidence of recurrence.

TABLE : 15 - DISTRIBUTION OF TRANSITIONAL CELL CARCINOMA AMONG THE VARIOUS CLINICOPATHOLOGICAL GROUPS FOR THE IHC STUDY (50 CASES)

Clinico-pathological factor		No. of cases (%)
Age	<66	37 (74%)
	>66	13 (26%)
Sex	Males	42 (84%)
	Females	8 (16%)
Site	Rt lateral wall	16 (32%)
	Lt lateral wall	13 (26%)
	Posterior wall	1 (2%)
	Base	4 (8%)
	Dome	5 (10%)
	Entire	6 (12%)
	Multiple	5 (10%)
Size	Mean size	4.32
Histological type	Papillary	45 (90%)
	Non-papillary	5 (10%)
Grade	Low	30 (60%)
	High	20 (40%)
Depth	Ta	8 (16%)
	T1	23 (46%)
	T2a	16 (32%)
	T2b	1 (2%)
	T3b	1 (2%)
	T4b	1 (2%)
Squamous metaplasia	Present	9 (18%)
	Absent	41 (82%)
Necrosis	Present	8 (16%)
	Absent	42 (84%)
Sarcomatous component	Present	1 (2%)
	Absent	49 (98%)
Stage	0	8 (16%)
	I	23 (46%)
	II	17 (34%)
	III	2 (4%)
Urine cytology	Positive	8 (66.7%)
	Negative	4 (33.3%)
Recurrence	present	19 (38%)
	Absent	31 (62%)

In this study, various prognostic factors were compared with mean microvessel density and HER-2/neu expression. The microvessel density ranged from 8 to 102 with a mean value of 36.44. 22 cases (44%) showed strong expression for HER-2/neu (scoring 2+ and 3+) and were considered positive and 28 cases (56%) showed weak expression (scoring 0+ and 1+) and were considered as negative (Table 16 & Chart 13).

TABLE : 16 - DISTRIBUTION OF HER-2/NEU EXPRESSION AND MICROVESSEL DENSITY IN TRANSITIONAL CELL CARCINOMA

IHC parameters	Result
MVD	Mean- 36.44
HER-2/neu	Positive-22(44%).
	Negative-28(56%)

CORRELATION OF MICROVESSEL DENSITY WITH VARIOUS CLINICO – PATHOLOGICAL PARAMETERS

In this study, the mean microvessel density for the male patients was found to be 38.45 with the standard deviation of 18.0. The mean MVD for the female patients was found to be 25.88 with the standard deviation of 6.3. Since the standard deviation was found to be very high, the logarithmic transformations was carried out before applying the student's t-test. The significant P-value infers that male patients have higher MVD than female patients (Table 17 and chart 14).

TABLE : 17 - CORRELATION OF GENDER WITH MICROVESSEL DENSITY

Gender	N	Mean MVD	Standard deviation(SD)	t-value*	P-value
Male	42	38.45	18.002	2.047	0.046
Female	8	25.88	6.379		

- **For the logarithmic values of MVD the t-test has been applied.**

This study showed that there is no statistically significant correlation between MVD and site of tumour. Since only one patient had tumour at the posterior wall, for statistical purpose its MVD value was combined with that of the base. The mean values have been compared using ANOVA test. The non-significant p-value infers that the site of the occurrence of the tumour has no influence on the MVD level. (Table 18).

TABLE : 18 - CORRELATION OF TUMOUR SITE WITH MICROVESSEL DENSITY

Site	N	Mean MVD	SD	ANOVA F-value*	P-value
Right lateral wall	16	32.69	9.884	0.472	0.795
Left lateral wall	13	37.54	20.630		
Base & Posterior wall	5	40.40	18.756		
Dome	5	30.60	13.722		
Entire bladder	6	40.83	30.407		
Multiple	5	42.20	11.256		

- **For the logarithmic values of MVD the ANOVA test has been applied.**

This study showed increased mean MVD in patients with multiple tumours (42.2±17.8) when compared to patients having single tumours (35.8±17.8). However, this increase was found to be statistically insignificant. (Table 19 and chart 15)

TABLE : 19 - CORRELATION OF TUMOUR NUMBER WITH MICROVESSEL DENSITY

Number	N	Mean MVD	SD	t-value	P value
Single	45	35.80	17.801	1.143	0.259
Multiple	5	42.20	11.256		

In this study, out of 50 patients, 29 of them had tumour size less than or equal to 4 cms. Their mean MVD level was 38.1 with the standard deviation of 17.0. Remaining 21 patients had tumour size above 4 cms and their mean MVD level was 34.05. The non-significant p-value infers that MVD is independent of the tumour size. (Table 20).

TABLE : 20 - CORRELATION OF TUMOUR SIZE WITH MICROVESSEL DENSITY

Size	N	Mean MVD	SD	t-value	P value
<4 cm	29	38.17	17.046	0.787	0.435
≥4 cm	21	34.05	17.735		

This study showed mean MVD of 34.89±15.7 in case of papillary tumours and 50.4±25.9 in case of non-papillary tumours. This increase was found to be statistically significant. (Table 21 and chart 16).

TABLE : 21 - CORRELATION OF HISTOLOGICAL TYPE WITH MICROVESSEL DENSITY

Histological type	N	Mean MVD	SD	t-value	P-value
Papillary	45	34.89	15.712	1.845	0.071
Non- papillary	5	50.40	25.929		

This study showed an increase in MVD in high grade carcinoma patients (38.6±24.4) in comparison with low grade patients (35±10.5). However, this difference was not found to be statistically significant and hence MVD was not influenced by the grade of the tumour. (Table 22 and chart 17)

TABLE : 22 - CORRELATION OF TUMOUR GRADE WITH MICROVESSEL DENSITY

Grade	N	Mean MVD	SD	t-value	P-value
Low grade	30	35.00	10.518	0.184	0.855
High grade	20	38.60	24.356		

This study showed increased MVD in cases of superficial carcinomas (44.75±25.3) in comparison with infiltrative carcinomas

(34.86±15.2) and the increase in MVD in superficial carcinomas was not statistically significant. (Table 23)

TABLE : 23 - CORRELATION OF INFILTRATION WITH MICROVESSEL DENSITY

T stage	N	Mean MVD	SD	t-value	P-value
Superficial	8	44.75	25.336	1.351	0.183
Infiltrative	42	34.86	15.208		

This study showed mean MVD of 44.75±25.3 in stage 0 (non-infiltrative) patients, 36.88±17.9 in stage I patients, 32.44±10.9 in stage II patients and 30±11.3 in stage III patients. Thus there was no increase in MVD in patients of increasing stage and this had no statistical significance. (Table 24 and chart 18)

TABLE : 24 - CORRELATION OF TNM STAGE WITH MICROVESSEL DENSITY

Stage	N	Mean MVD	SD	ANOVA –F value	P-value
0	8	44.75	25.336	0.700	0.557
I	23	36.88	17.858		
II	17	32.44	10.899		
III	2	30.00	11.314		

In this study, the mean MVD in TCC with squamous metaplasia was 29±11.8 and in those without squamous metaplasia was 38±17.9. This increase in MVD was statistically significant. The mean MVD in TCC with necrosis was 28.5±11.6 and in those without necrosis was 37.9±17.9. This increase in MVD was found to be statistically significant. The MVD in TCC with sarcomatoid component was 57 and the mean MVD in TCC without sarcomatoid component was 36±17.2. This decrease in value was not statistically significant (Table 25)

TABLE : 25 - CORRELATION OF MICROVESSEL DENSITY WITH OTHER PARAMETERS

Patient characteristics		N	Mean MVD	SD	t-value	P-value
Squamous metaplasia	Present	9	29.00	11.758	1.820	0.075
	Absent	41	38.07	17.973		
Necrosis	Present	8	28.50	11.588	1.844	0.071
	Absent	42	37.95	17.871		
Sarcomatoid component	Present	1	57.00	-	1.265	0.212
	Absent	49	36.02	17.202		

This study showed an increase in mean MVD in patients with positive urine cytology (44±25.5) than that of patients with negative urine cytology (25.8±5.2). However, this increase in value was not found to be statistically significant. (Table 26 and chart 19).

TABLE : 26 - CORRELATION OF URINE CYTOLOGY POSITIVITY WITH MICROVESSEL DENSITY

Urine cytology	N	Mean MVD	SD	t-value	P-value
Positive	8	44.00	25.489	1.766	0.108
Negative	4	25.75	5.188		

In this study, the patients with recurrence had an increased mean MVD value (39.9±18.4) than those without recurrence (33.9±16.3). However, this increase in MVD values was not statistically significant (Table 27 and chart 20).

TABLE : 27 - CORRELATION OF TUMOUR RECURRENCE WITH MICROVESSEL DENSITY

Recurrence	N	Mean MVD	SD	t-value	P-value
Present	19	41.73	18.409	1.333	0.189
Absent	31	33.19	16.281		

The present study showed that there was statistically significant association between microvessel density and histological type, male gender and necrosis. Microvessel density was seen to increase with increasing number, grade, presence of sarcomatoid component, urine cytology positive tumours and in recurrent tumours. But when subjected to statistical analysis this association was not found to be significant. There was an increased expression in large tumours involving the entire bladder.

CORRELATION OF HER-2/NEU WITH VARIOUS CLINICOPATHOLOGICAL FACTORS

In this study, the mean age of patients with positive HER-2/neu expression was 62.9±8.9 and the mean age of patients with negative HER-2/neu expression was 58.2±11. However, this increase in HER-2/neu expression with age was not found to be statistically significant (Table 28).

TABLE : 28 - CORRELATION OF AGE WITH HER-2/NEU EXPRESSION

HER-2/neu	No. of subjects	Mean age	SD	t-test value	P-value
Positive	22	62.95	8.904	1.649	0.106
Negative	28	58.18	11.046		

In this study, HER-2/neu positivity was noted in 45.2% of male patients and 37.5% of female patients. The non-significant p-value of the chi-square test shows that this association of increased HER-2/neu expression in males was not statistically significant (Table 29 and chart 21).

TABLE : 29 - CORRELATION OF GENDER WITH HER-2/NEU EXPRESSION

Gender	HER-2/neu positive (%)	HER-2/neu negative (%)	Total	Pearson chi square test	P-value
Male	19 (45.2%)	23 (54.8%)	42	0.163	0.686
Female	3 (37.5%)	5 (62.5%)	8		
Total	22 (44%)	28 (58%)	50		

In the present study, HER-2/neu positivity was observed in 50% of tumours arising from the right lateral wall, 46.2% of tumours of left lateral wall, 1% of tumours of the dome, 0% in tumours of base and posterior wall, 4% in tumours involving the entire bladder and 3% in cases of multiple tumours. But this is statistically insignificant. Thus there is no difference in HER-2/neu expression of tumours arising from different sites (Table 30).

TABLE : 30 - CORRELATION OF TUMOUR SITE WITH HER-2/NEU EXPRESSION

Site	HER-2/neu positive	HER-2/neu negative	Total	Pearson chi square test	P-value
Right lateral wall	8 (50%)	8 (50%)	16	7.126	0.211
Left lateral wall	6 (46.2%)	7 (53.8%)	13		
Base & Posterior wall	0 (0%)	5 (100%)	5		
Dome	1 (20%)	4 (80%)	5		
Entire	4 (66.7%)	2 (33.3%)	6		
Multiple	3 (60%)	2 (40%)	5		
Total	22 (44%)	28 (56%)	50		

In this study, HER-2/neu positivity was noted in 42.2% of single tumours and 60% in cases of multiple tumours. However this increase in HER-2/neu expression was not found to be statistically significant (Table 31 and Chart 22).

TABLE : 31 - CORRELATION OF TUMOUR NUMBER WITH HER-2/NEU EXPRESSION.

Number	HER-2/neu positive	HER-2/neu negative	Total	Pearson chi square test	P-value
Single	19 (42.2%)	26 (57.8%)	45	0.577	0.447
Multiple	3 (60%)	2 (40%)	5		
Total	22	28	50		

This study showed 41.4% HER-2/neu positivity in tumours < 4 cm and 47.6% positivity in tumours ≥ 4cm. However, this increase in HER-2/neu positivity with size was not found to be statistically significant (Table 32).

TABLE : 32 - CORRELATION OF TUMOUR SIZE WITH HER-2/NEU EXPRESSION

Size	HER-2/neu positive	HER-2/neu negative	Total	Pearson chi square test	P-value
<4 cm	12 (41.4%)	17 (58.6%)	29	0.192	0.661
≥4 cm	10 (47.6%)	11(52.4%)	21		
Total	22	28	50		

Among histological forms, 44.4% of papillary tumours and 40% of non-papillary tumours showed HER-2/neu positivity. However, this increase in HER-2/neu positivity in papillary tumours was not statiatically significant (Table 33 and Chart 23).

TABLE : 33 - CORRELATION OF HISTOLOGICAL TYPE WITH HER-2/NEU EXPRESSION

Histological type	HER-2/neu positive	HER-2/neu negative	Total	Pearson chi square test	P-value
Papillary	20 (44.4%)	25 (55.6%)	45	0.036	0.849
Non-papillary	2 (40%)	3 (60%)	5		
Total	22	28	50		

In this study, an increasing percentage of cases showing HER-2/neu positivity in high grade tumours were observed. 30% of low grade tumours and 65% of high grade tumours showed positivity for HER-2/neu. This association was found to be statistically significant (Table 34 and Chart 24).

TABLE : 34 - CORRELATION OF TUMOUR GRADE WITH p53 EXPRESSION

Grade	HER-2/neu positive	HER-2/neu negative	Total	Pearson chi square test	P-value
Low	9 (30%)	21 (70%)	30	5.96	0.015
High	13 (65%)	7 (35%)	20		
Total	22	28	50		

In this study, an increase in HER-2/neu expression was seen in infiltrative tumours (47.6%) when compared to superficial tumours (25%). However, this association was not found to be statistically significant (Table 35).

TABLE : 35 - CORRELATION OF INFILTRATION WITH HER-2/NEU EXPRESSION

T stage	HER-2/neu positive	HER-2/neu negative	Total	Pearson chi square test	P-value
Superficial	2 (25%)	6 (75%)	8	1.395	0.238
Infiltrative	20 (47.6%)	22 (52.4%)	42		
Total	22	28	50		

HER-2/neu positivity was noticed in 25% of stage 0 cases, 37.5% of stage I cases, 62.5% of stage II cases and 37.5% of Stage III cases.

There is increase in HER-2/neu expression with increase in stage except for stage III where there was only one HER-2/neu positive case and one HER-2/neu negative case. However, this association was not found to be statistically significant (Table 36 and Chart 25).

TABLE : 36 - CORRELATION OF TNM STAGE WITH HER-2/NEU EXPRESSION

T stage	HER-2/neu positive	HER-2/neu negative	Total	Pearson chi square test	P-value
0	2 (25%)	6 (75%)	8	3.835	0.280
I	9 (37.5%)	15 (62.5%)	24		
II	10 (62.5%)	6 (37.5%)	16		
III	1 (50%)	1 (50%)	2		
Total	22	28			

In this study, HER-2/neu positivity was noted in 33.3% of cases with squamous metaplasia, 62.5% cases with necrosis, 100% cases with evidence of sarcomatoid component. However, none of the values were found to be statistically significant using Pearson's chi square test and the p-value was found to be >0.05 (Table37).

TABLE : 37 - CORRELATION OF HER-2/NEU WITH OTHER PROGNOSTIC PARAMETERS

Patient characteristics		HER-2/neu positive	HER-2/neu negative	Total	Pearson chi square test	P-value
Squamous metaplasia	Present	3(33.3%)	6(66.7%)	9	0.507	0.477
	Absent	19(46.3%)	22(53.7%)	41		
Necrosis	Present	5(62.5%)	3(37.5%)	8	1.323	0.250
	Absent	17(40.5%)	25(59.5%)	42		
Sarcomatoid component	Present	1(100%)	0	1	1.299	0.254
	Absent	21(42.9%)	28(57.1%)	49		

In this study, HER-2/neu was positive in 75% of urine cytology positive tumours and 25%of urine cytology negative tumours. This increase in HER-2/neu expression in cases of positive urine cytology was found to be statistically significant. (Table 38 and chart 26).

TABLE : 38 - CORRELATION OF URINE CYTOLOGY POSITIVITY WITH HER-2/NEU EXPRESSION

Urine cytology	HER-2/neu positive	HER-2/neu negative	Total	Pearson chi-square test	P-value
Positive	6 (75%)	2 (25%)	8	2.743	0.098
Negative	1 (25%)	3 (75%)	4		
Total	7	5	12		

In this study, HER-2/neu expression was found to be positive in 47.4% of recurrent tumours and 41.9 of non-recurrent tumours. However, this increase in HER-2/neu expression in recurrent tumours was not found to be statistically significant (Table 39 and chart 27).

TABLE : 39 - CORRELATION OF TUMOUR RECURRENCE WITH HER-2/NEU EXPRESSION

Recurrence	HER-2/neu positive	HER-2/neu negative	Total	Pearson chi square test	P-value
Yes	9 (47.4%)	10 (52.6%)	19	1.032	0.310
No	13 (41.9%)	18 (58.1%)	31		
Total	22	28	50		

This study showed that there was a statistically significant association between HER-2/neu overexpression in case of high grade tumours and urine cytology positive tumours. There was an increased expression in male gender and multiple tumours. HER-2/neu over-expression was seen to increase with increasing age, size, depth of infiltration, TNM stage, presence of necrosis, sarcomatoid component and in cases of recurrent tumours. But when subjected to statistical analysis this association was found to be statistically insignificant.

CORRELATION OF HER-2/NEU EXPRESSION WITH MICROVESSEL DENSITY

The mean MVD in HER-2/neu positive tumours was 36.95+22.4 whereas the mean MVD in HER-2/neu negative tumours was 36.04+12.3. This minimal increase in MVD values in case of HER-2/neu positive tumours was not statistically significant and hence there is no difference in mean MVD values in tumours with variable HER-2/neu expression (Table 40 and chart 28).

TABLE : 40 - MEAN MVD IN HER-2/NEU POSITIVE AND NEGATIVE TUMOURS

HER-2/neu	Number of cases	Mean MVD	SD	t-value	P-value
Positive(%)	22	36.95	22.39	0.434	0.667
Negative(%)	28	36.04	12.32		

CHART 1 - HISTOLOGICAL SUBTYPES OF BLADDER CARCINOMAS

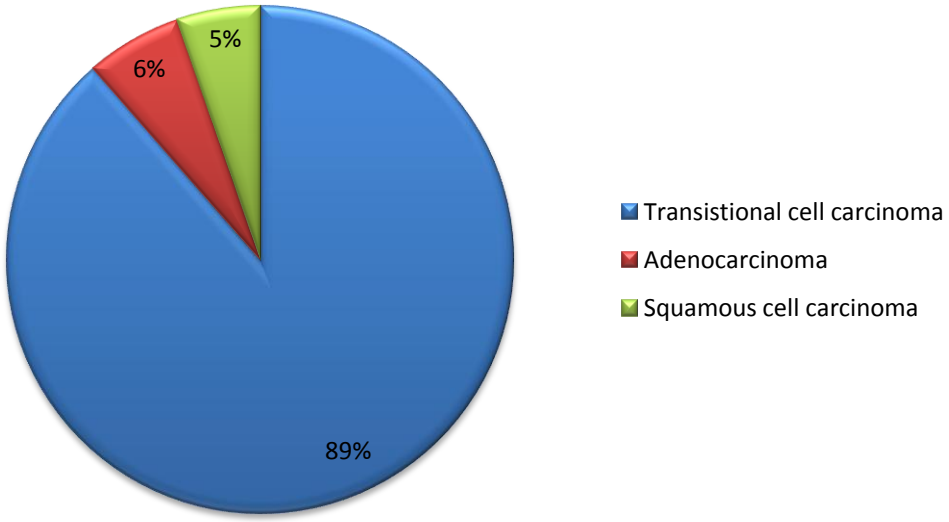


CHART 2 - AGE WISE DISTRIBUTION OF TRANSITIONAL CELL CARCINOMAS

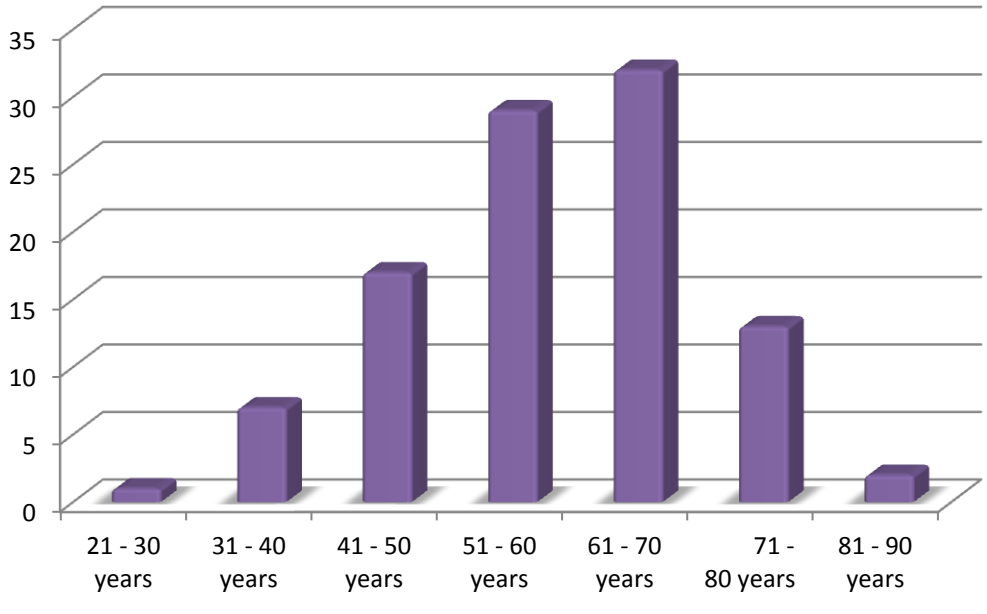


CHART 3 - SEX DISTRIBUTION IN TRANSITIONAL CELL CARCINOMAS

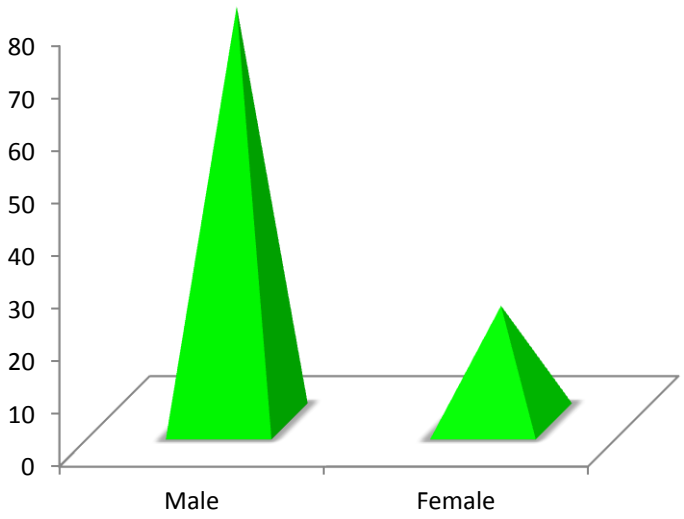


CHART 4 - SITE OF INVOLVEMENT IN TRANSITIONAL CELL CARCINOMAS

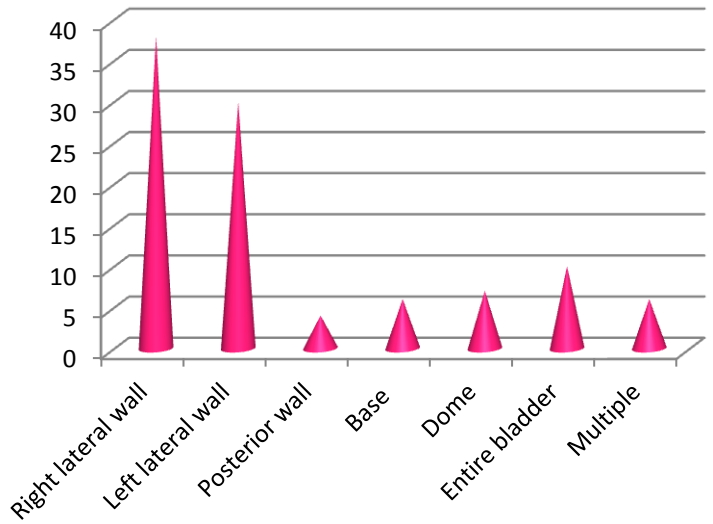


CHART 5 - TUMOUR NUMBER IN TRANSITIONAL CELL CARCINOMAS

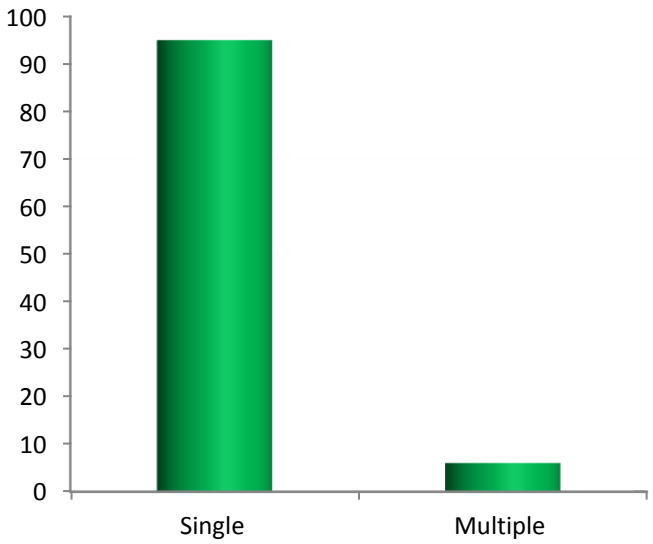


CHART 6 - SIZE IN TRANSITIONAL CELL CARCINOMAS

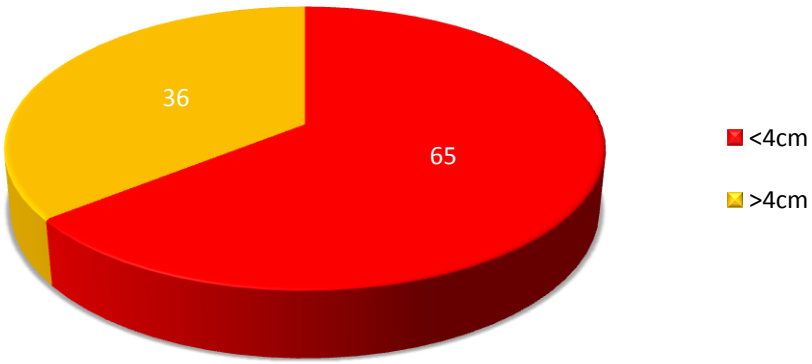


CHART 7 - HISTOLOGICAL APPEARANCE OF TRANSITIONAL CELL CARCINOMAS

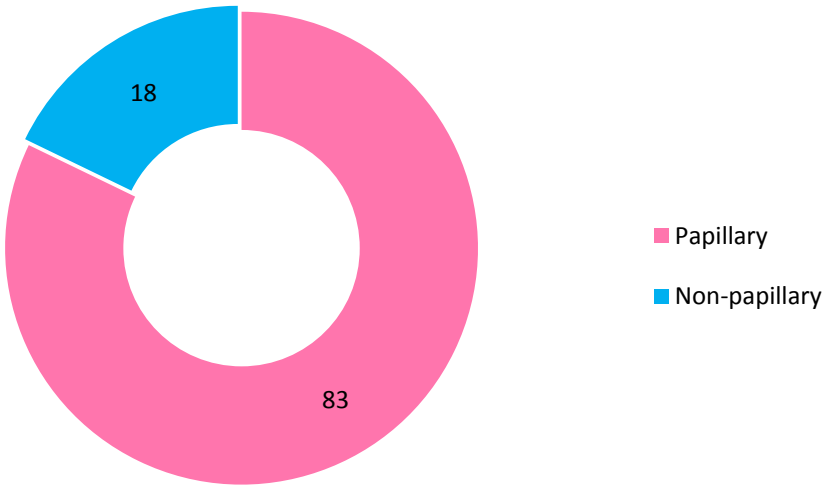


CHART 8 - HISTOLOGICAL GRADE IN TRANSITIONAL CELL CARCINOMAS

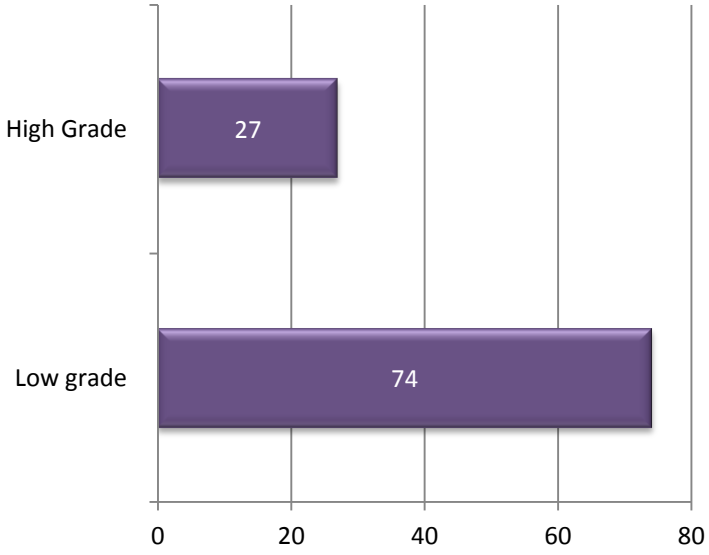


CHART 9 - DISTRIBUTION OF TRANSITIONAL CELL CARCINOMAS ACCORDING TO STAGE

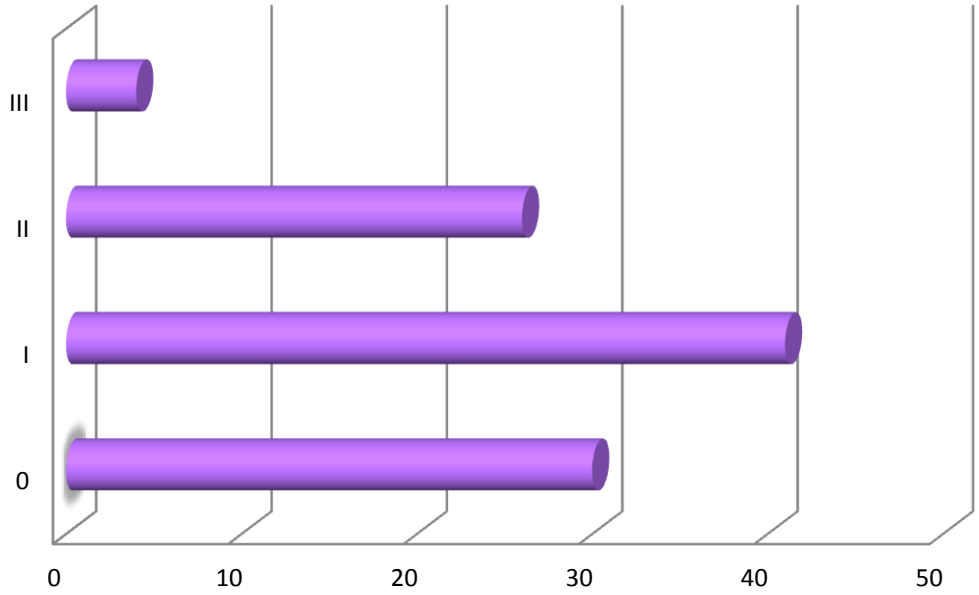


CHART 10 - DISTRIBUTION OF OTHER PROGNOSTIC FACTORS IN TRANSITIONAL CELL CARCINOMA

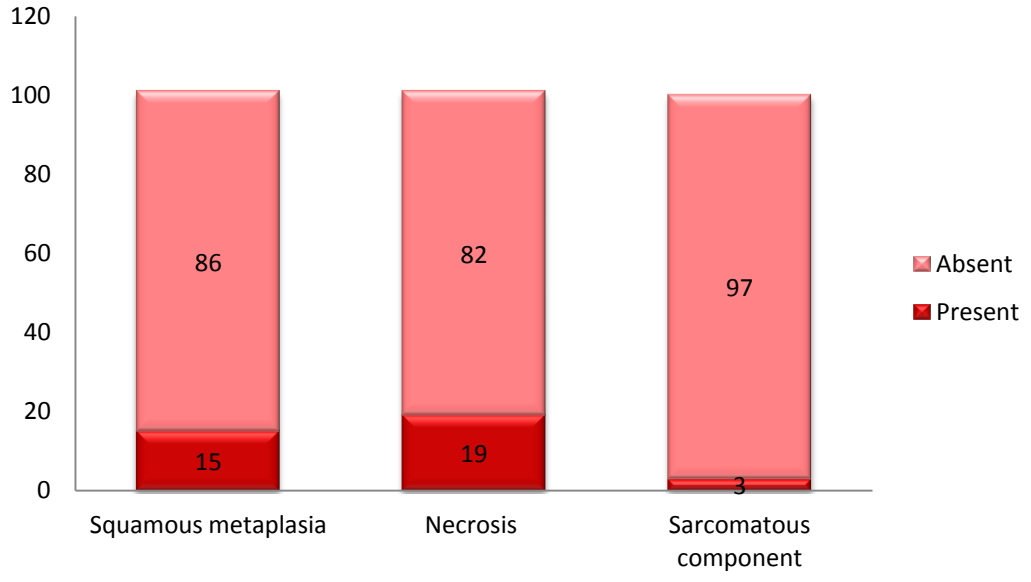


CHART 11 - URINE CYTOLOGY IN TRANSITIONAL CELL CARCINOMAS

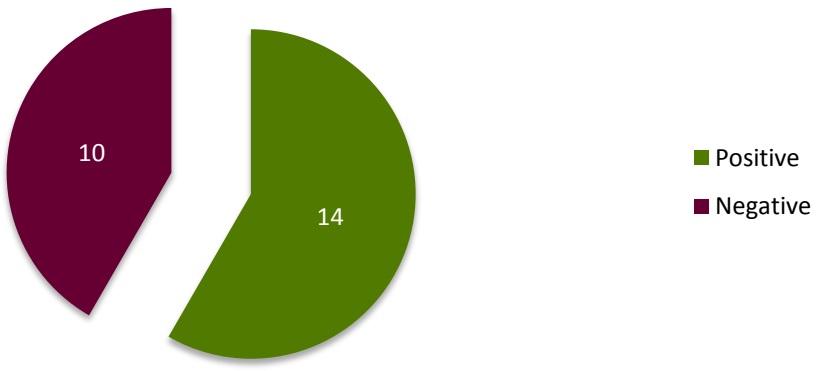


CHART 12 - RECURRENCE IN TRANSITIONAL CELL CARCINOMAS

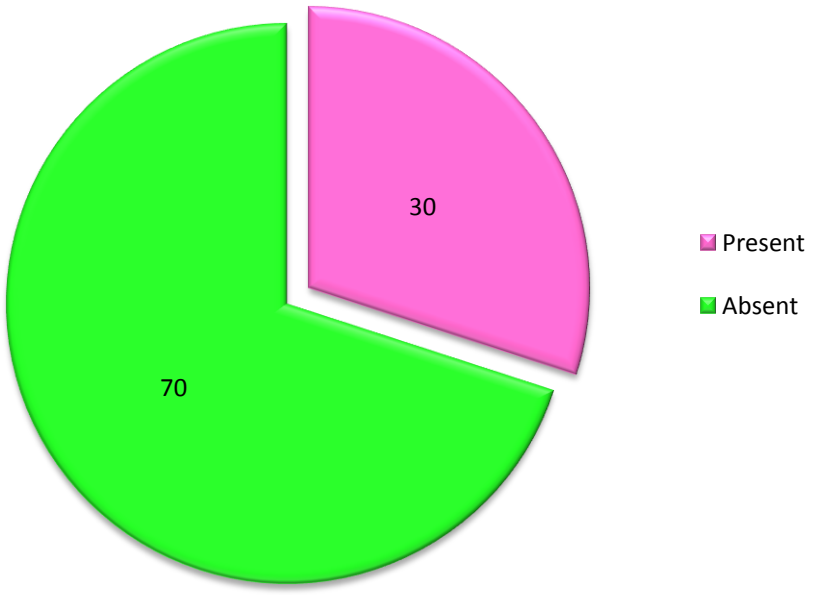


CHART 13 - DISTRIBUTION OF HER-2 NEU EXPRESSION AND MICROVESSEL DENSITY IN TRANSITIONAL CELL CARCINOMAS

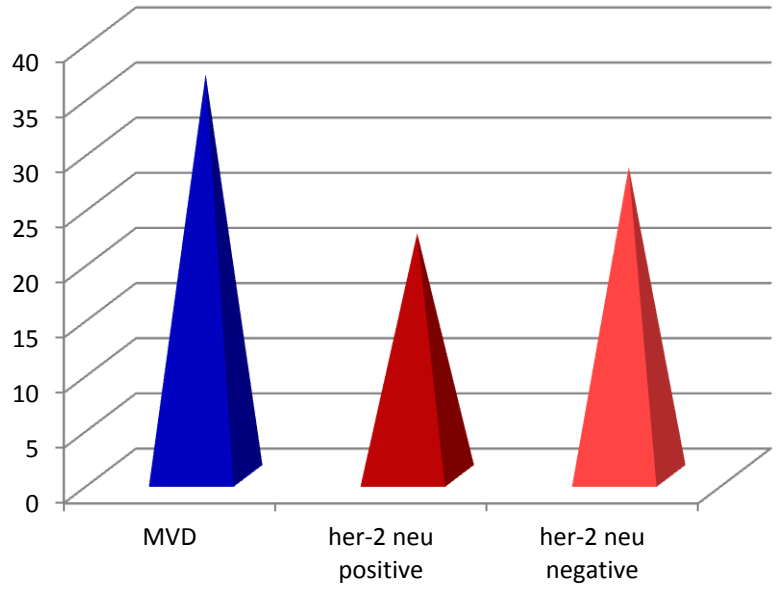


CHART 14 - GENDER VS MVD

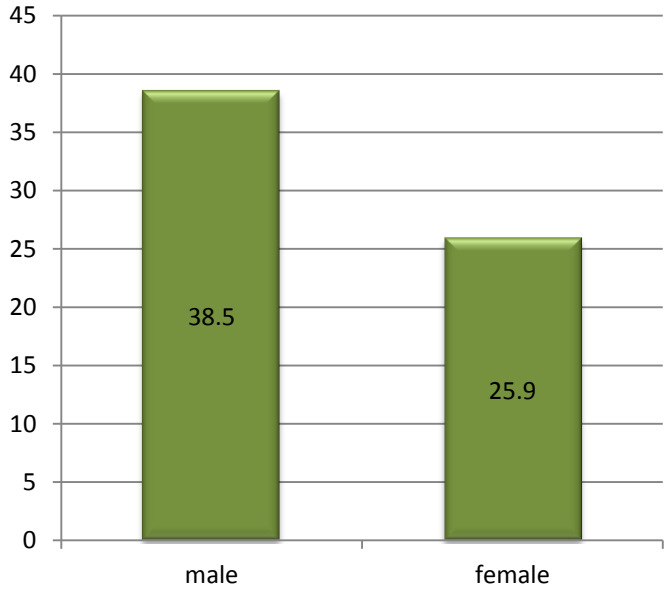


CHART 15 - TUMOUR NUMBER VS MVD

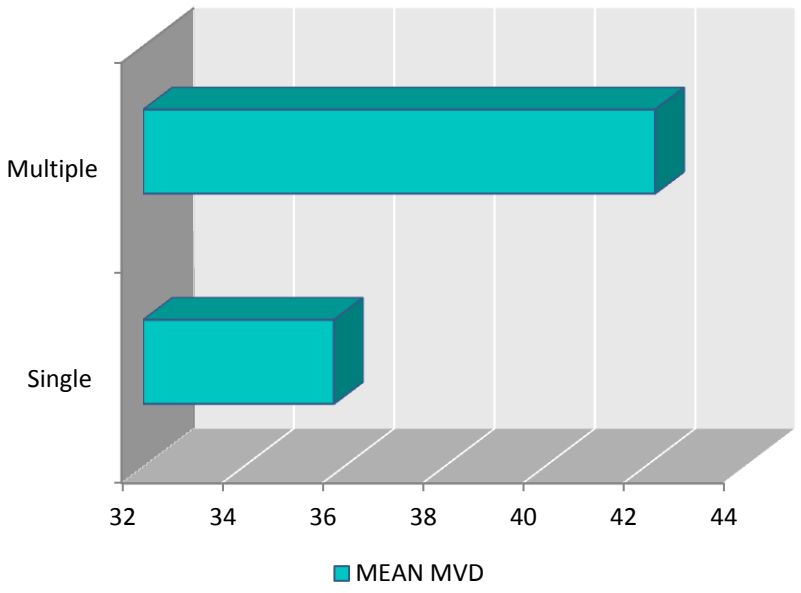


CHART 16 - HISTOLOGICAL TYPE VS MVD

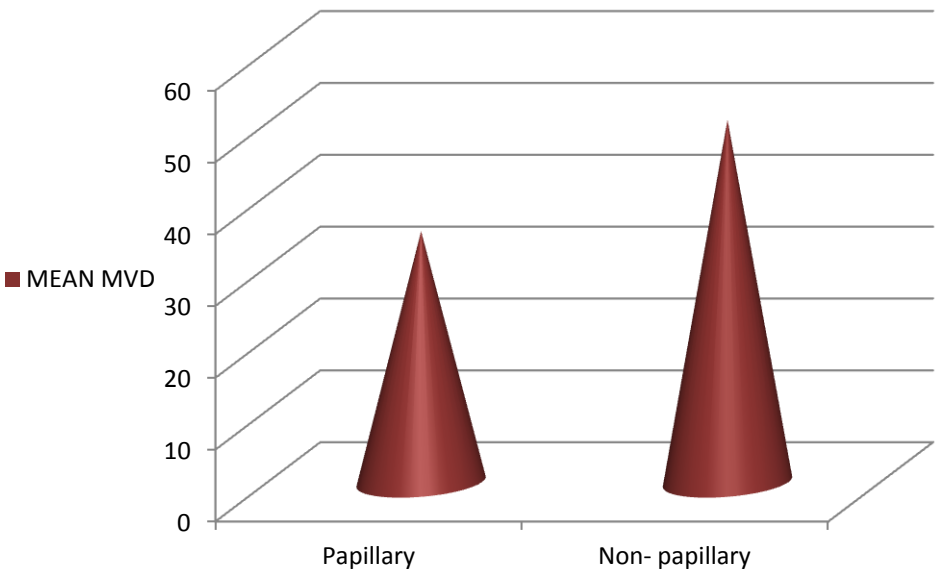


CHART 17 - GRADE VS MVD

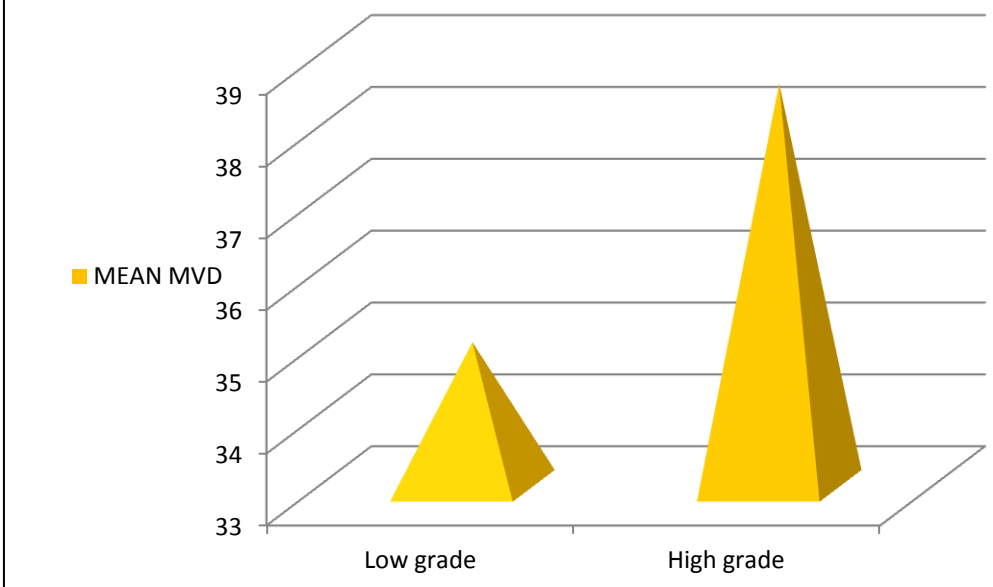


CHART 18 - STAGE VS MVD

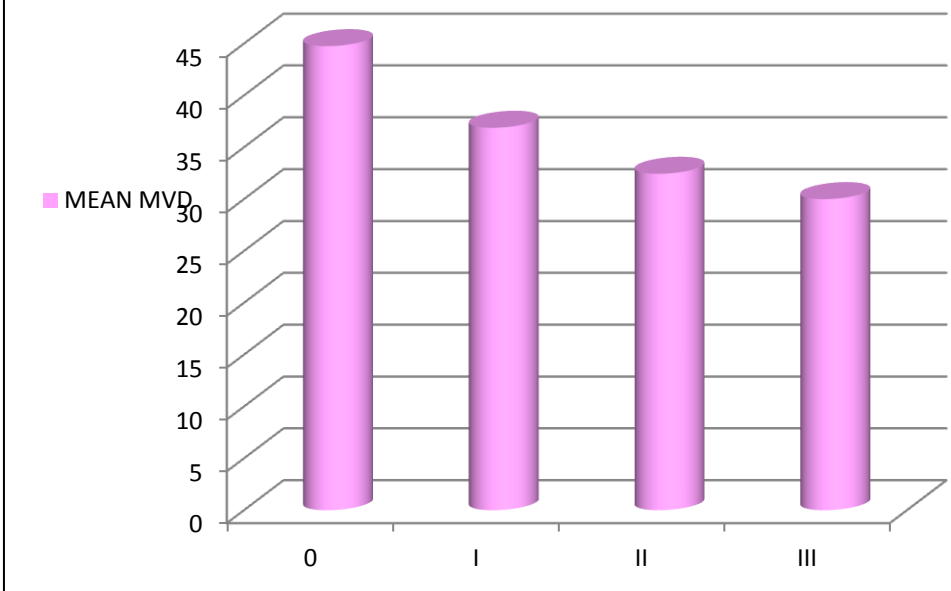


CHART 19 - URINE CYTOLOGY VS MVD

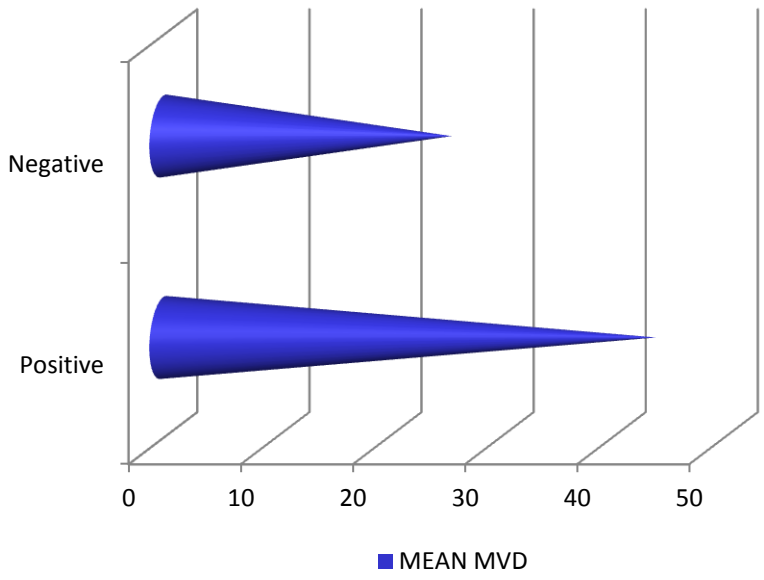


CHART 20 - RECURRENCE VS MEAN MVD

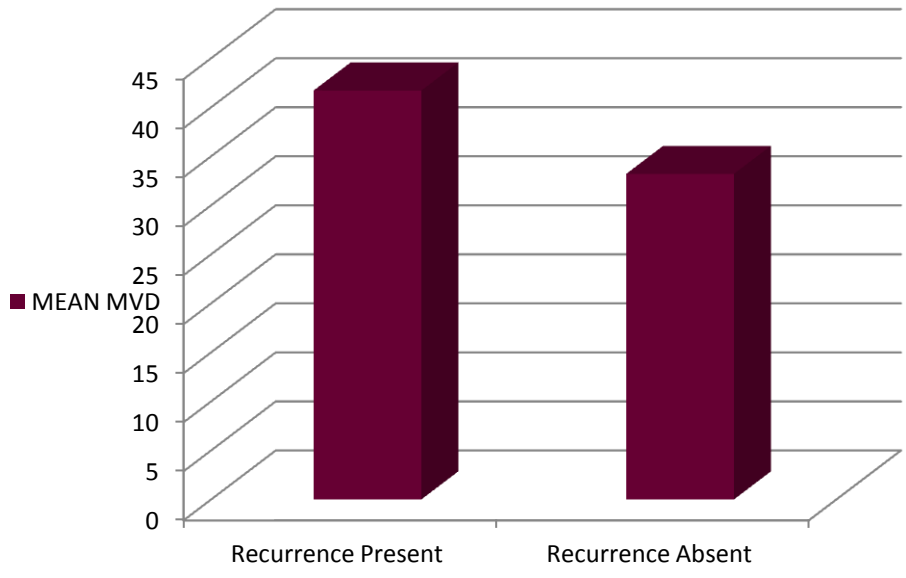


CHART 21 - GENDER VS HER-2 NEU EXPRESSION

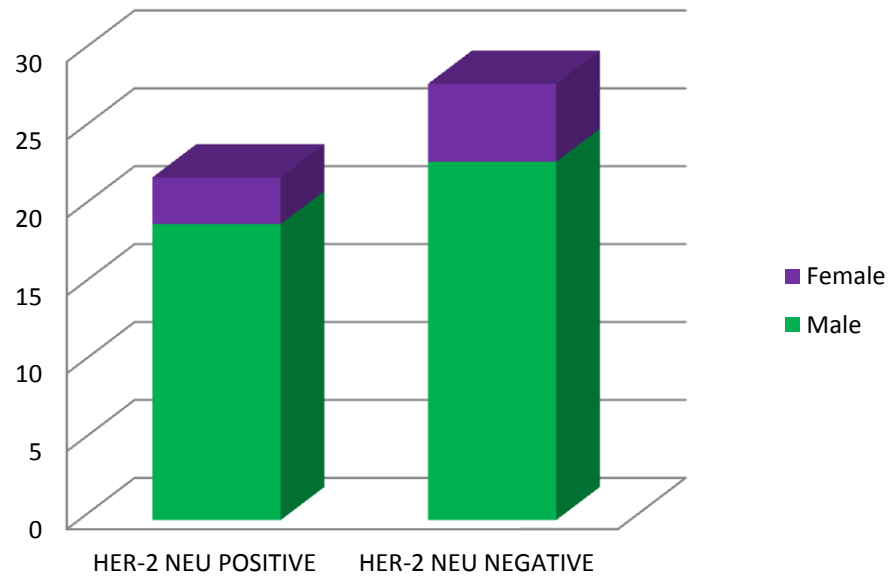


CHART 22 - TUMOUR NUMBER VS HER-2 NEU EXPRESSION

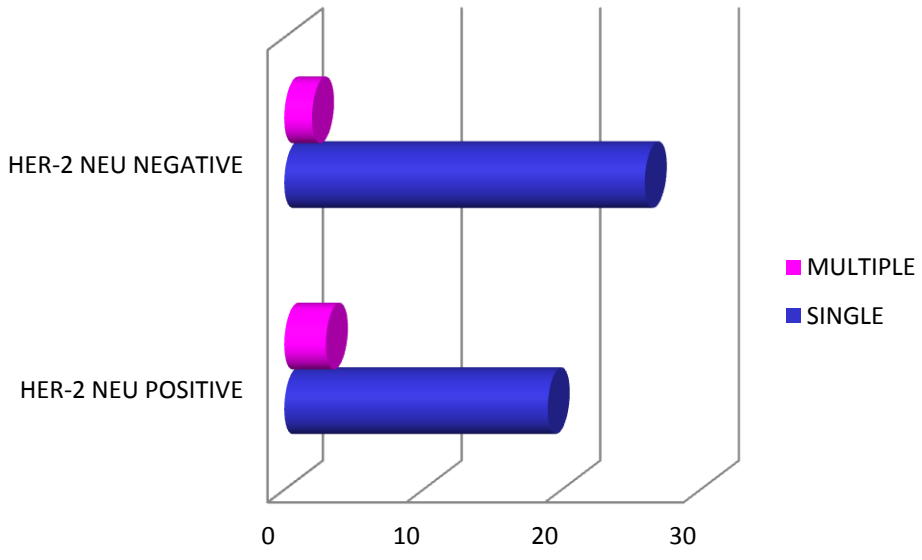


CHART 23 - HISTOLOGICAL TYPE VS HER-2 NEU EXPRESSION

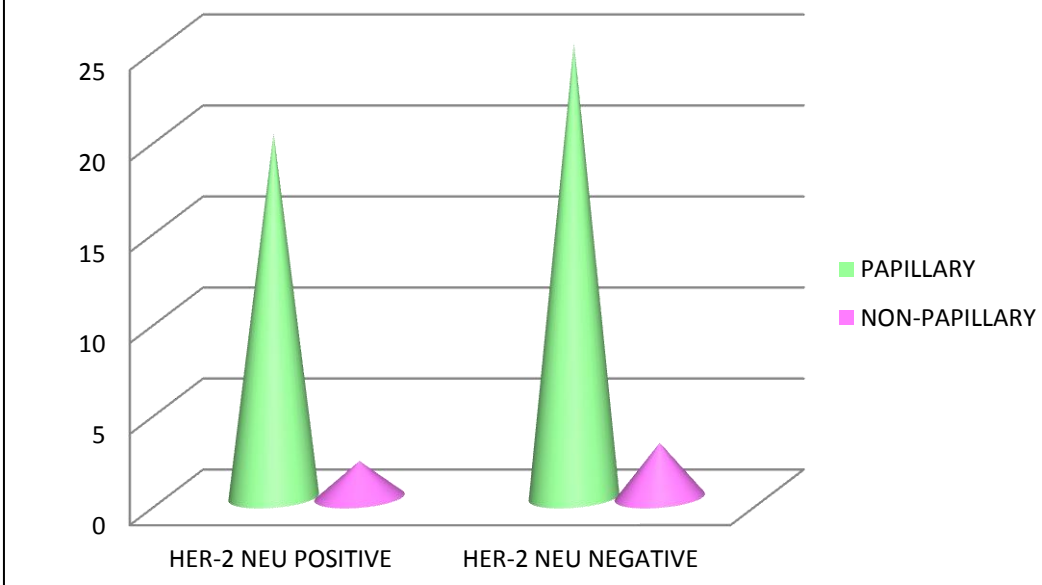


CHART 24 - GRADE VS HER-2 NEU EXPRESSION

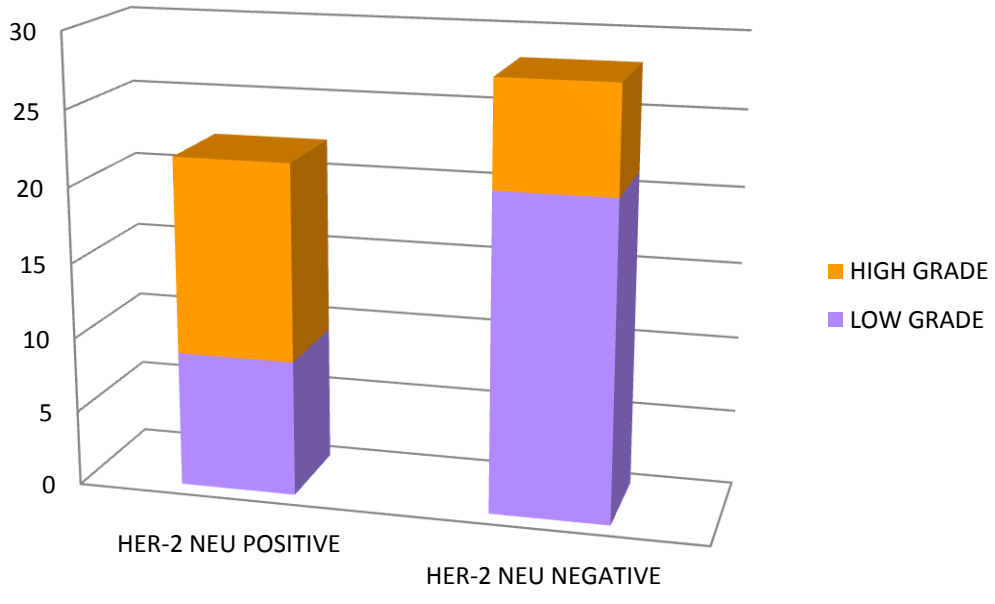


CHART 25 - STAGE VS HER-2 NEU EXPRESSION

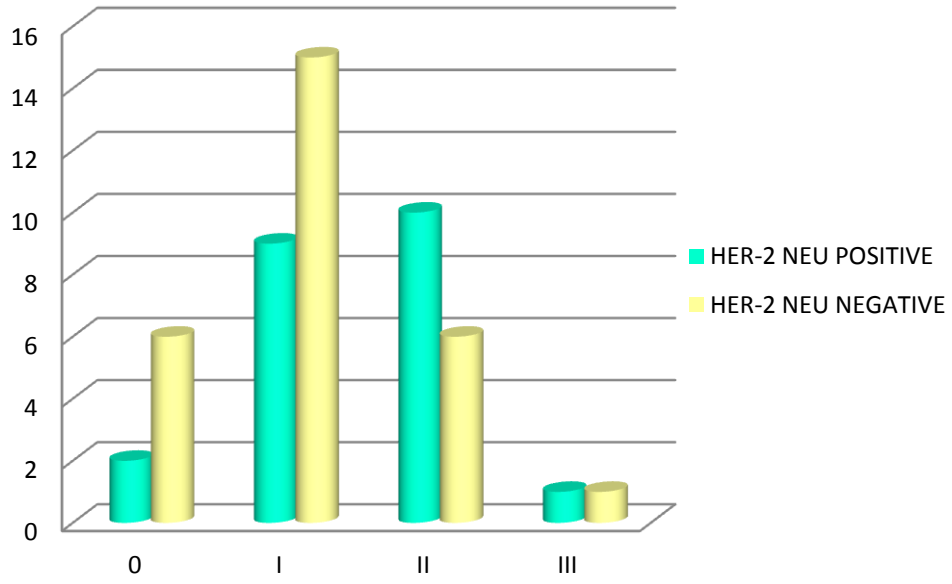


CHART 26 - URINE CYTOLOGY VS HER-2 NEU EXPRESSION

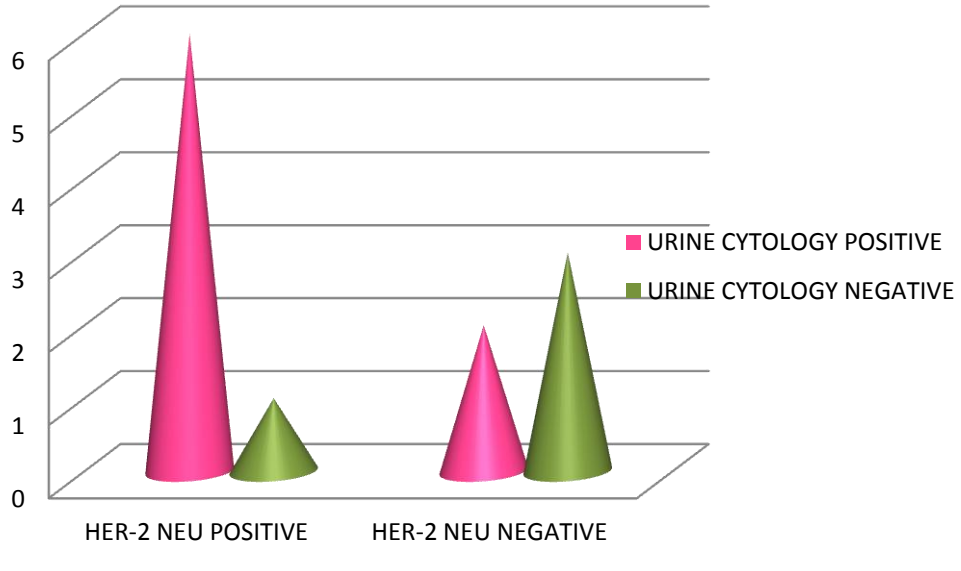


CHART 27 - RECURRENCE VS HER-2 NEU EXPRESSION

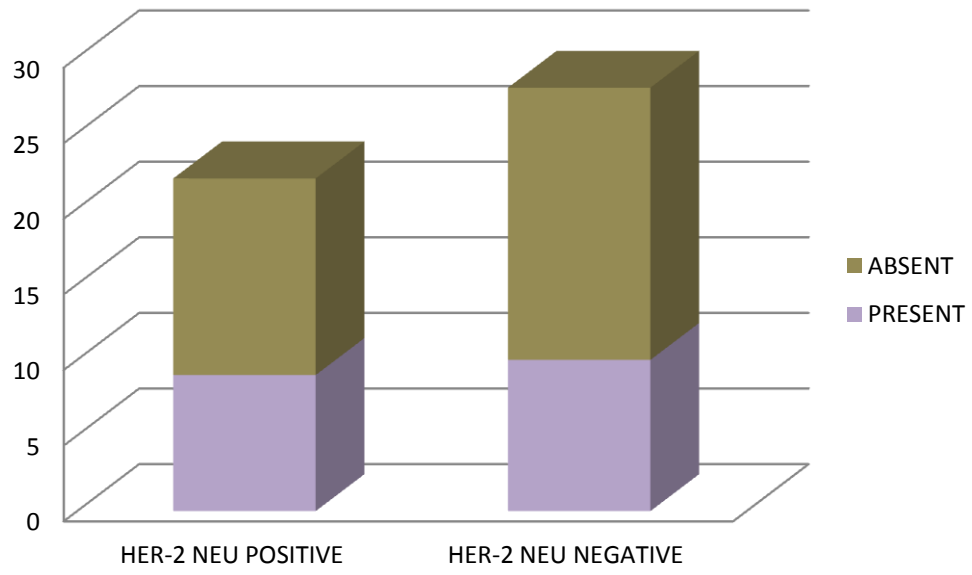
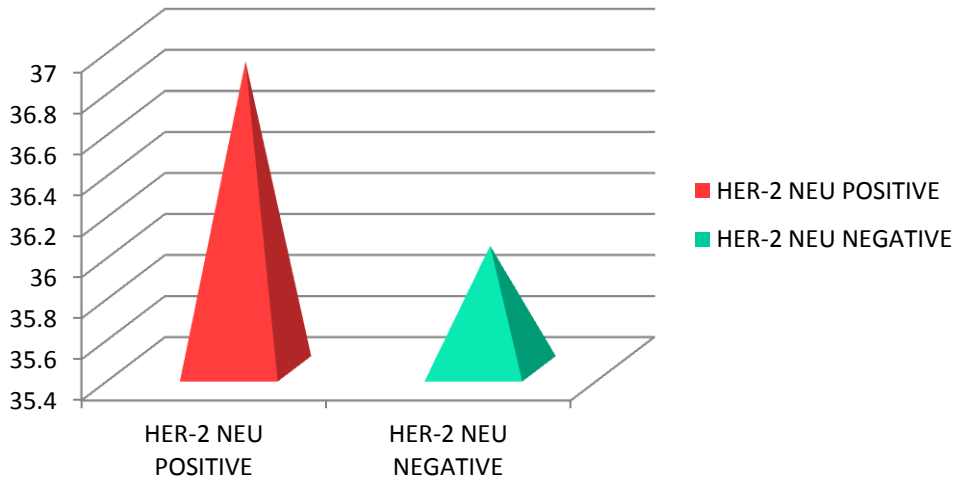


CHART 28 - MEAN MVD IN HER-2 NEU POSITIVE AND NEGATIVE TUMOURS



TRANSITIONAL CELL CARCINOMA OF BLADDER- LOW GRADE

FIGURE 3: Transitional cell carcinoma of bladder- papillary type

TRANSITIONAL CELL CARCINOMA OF BLADDER- HIGH GRADE

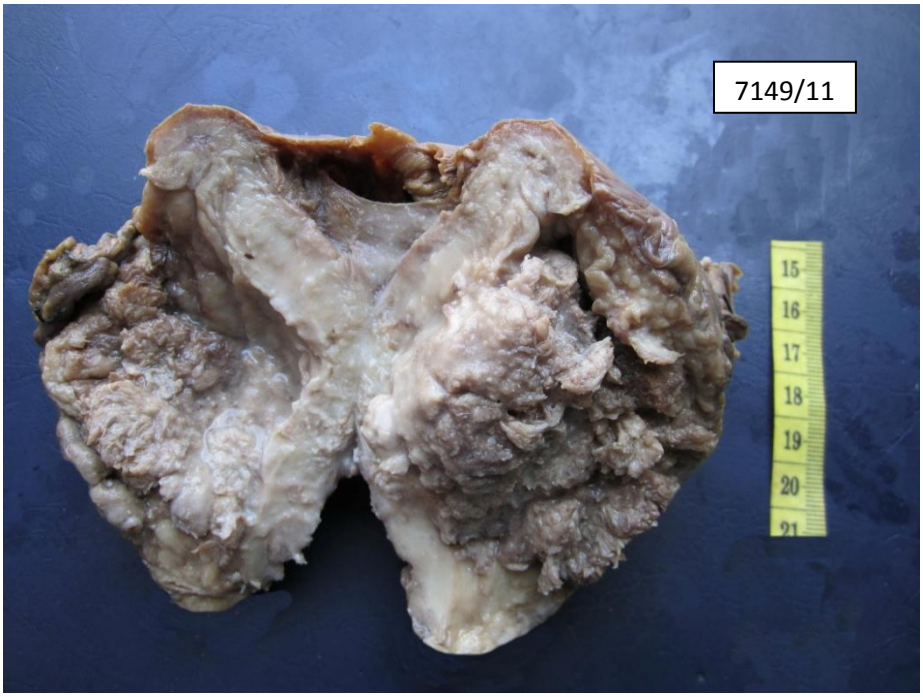


FIGURE 4: Transitional cell carcinoma of bladder involving entire bladder

SQUAMOUS CELL CARCINOMA OF BLADDER

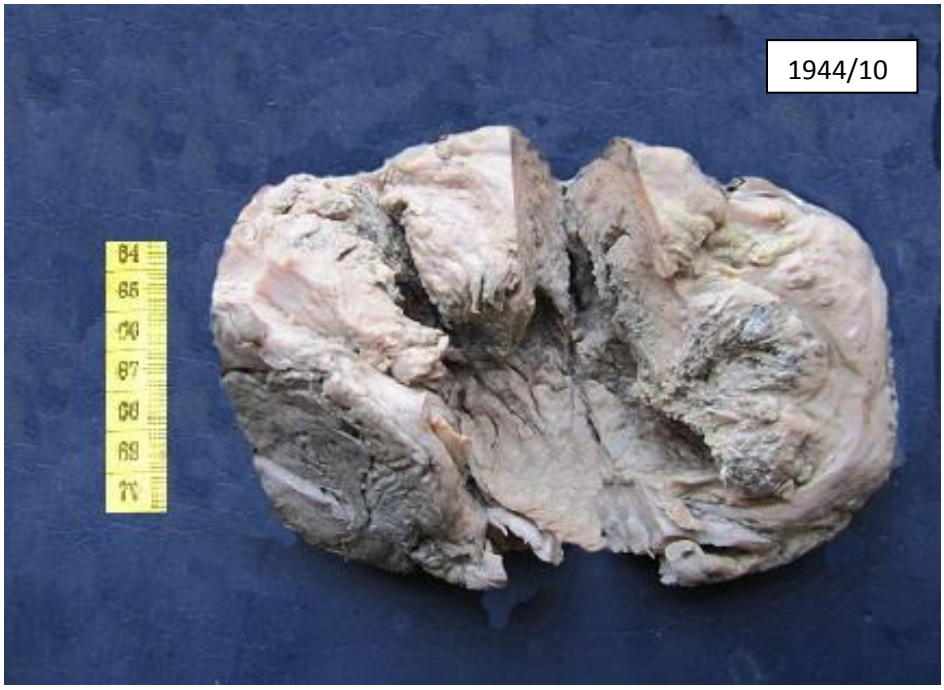


FIGURE 5: Growth involving the entire bladder

ADENOCARCINOMA OF BLADDER

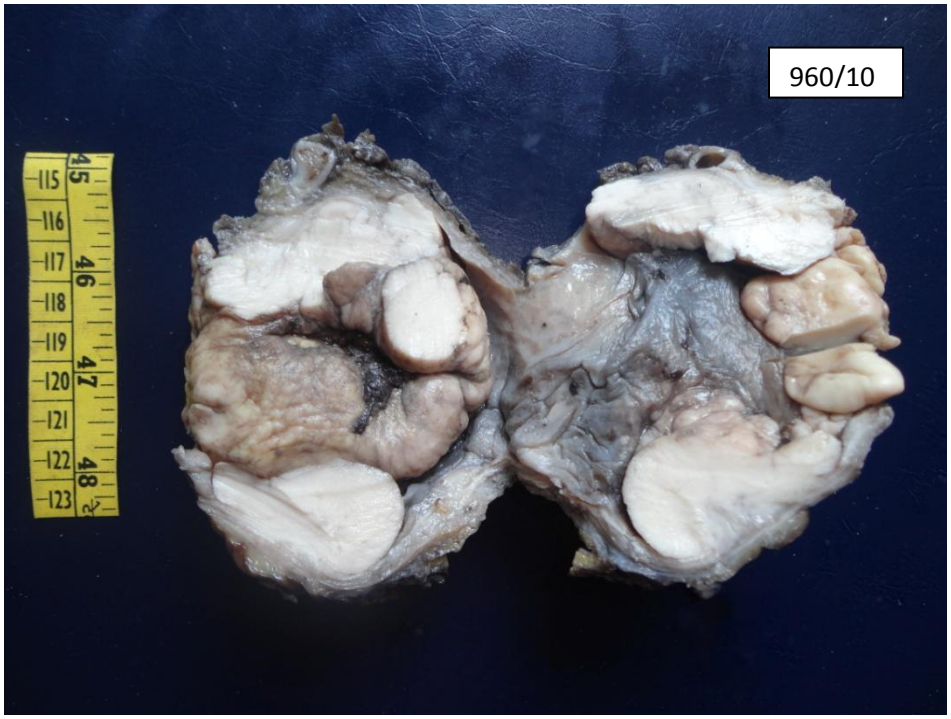


FIGURE 6: Multicentric growth within bladder

PAPILLARY TRANSITIONAL CELL CARCINOMA- LOW GRADE

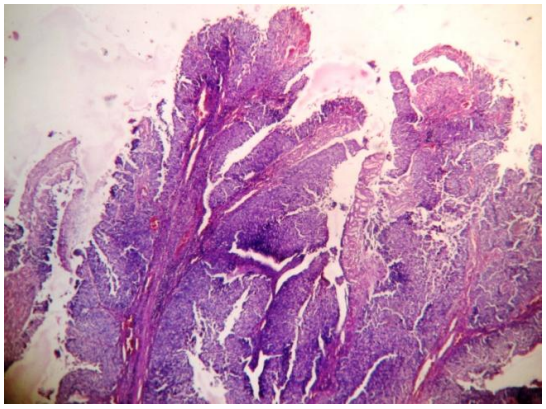


FIGURE 7: Low grade transitional cell carcinoma with cells arranged in papillary pattern (100X) HPE 7933/11

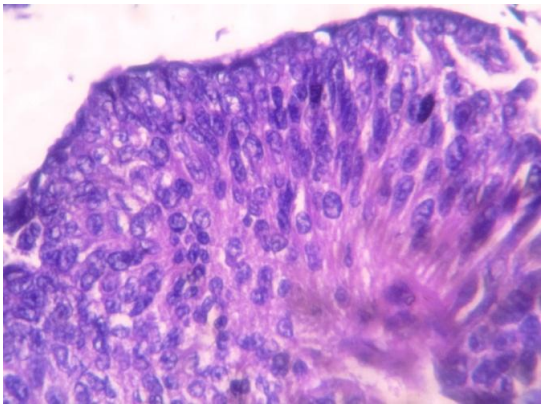


FIGURE 8: shows 10 layers of epithelial cells with mild nuclear atypia. Umbrella cells are preserved (400X) HPE 7933/11

PAPILLARY TRANSITIONAL CELL CARCINOMA- HIGH GRADE

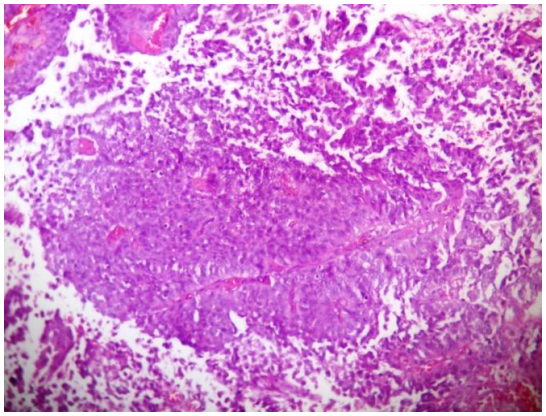


FIGURE 9: High grade transitional cell carcinoma with cells arranged in papillary pattern (100X) HPE 5743/11

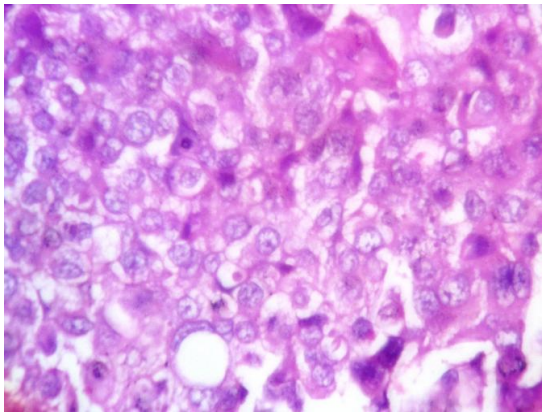


FIGURE 10: Malignant epithelial cells showing loss of polarity, pleomorphism and prominent nucleoli (400X) HPE 5743/11

NON-PAPILLARY TRANSITIONAL CELL CARCINOMA

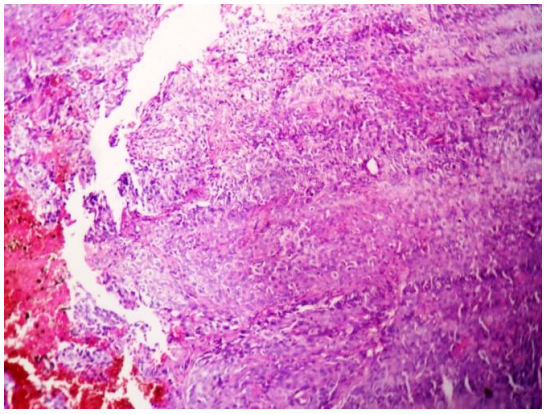


FIGURE 11: Non papillary transitional cell carcinoma with cells arranged in solid sheets (100X) HPE 71/11

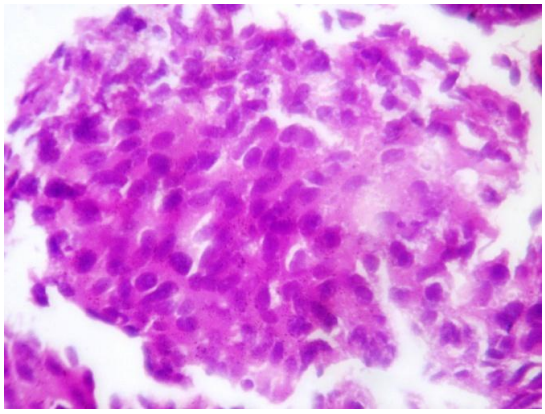


FIGURE 12: shows a high grade neoplasm with nuclear pleomorphism, loss of polarity and loss of umbrella cells (400X) 71/11

INFILTRATION IN TRANSITIONAL CELL CARCINOMA

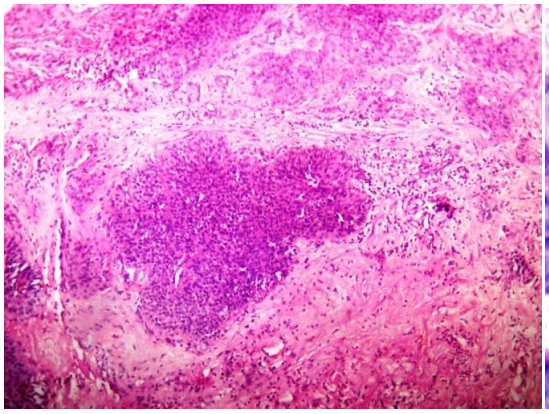


FIGURE 13: shows infiltration of transitional cell carcinoma into underlying submucosa (100X) HPE 4868/11

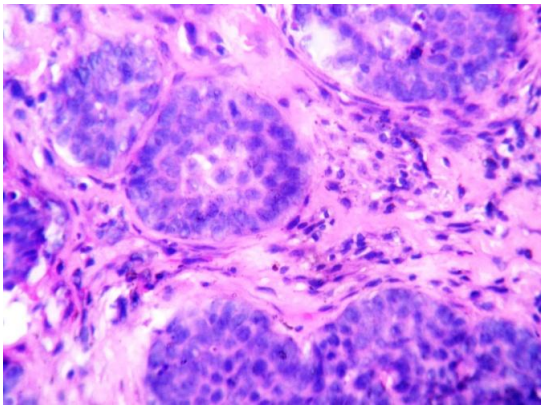


FIGURE 14: shows infiltration of malignant epithelial cells into underlying muscle layer (400X) HPE 4846/11

OTHER PROGNOSTIC FACTORS

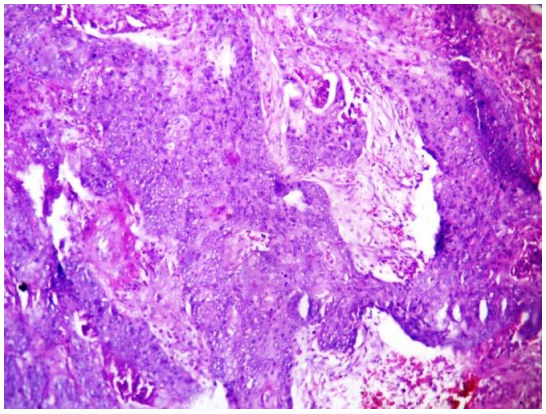


FIGURE 15: Squamous metaplasia (100X) HPE 6090/11

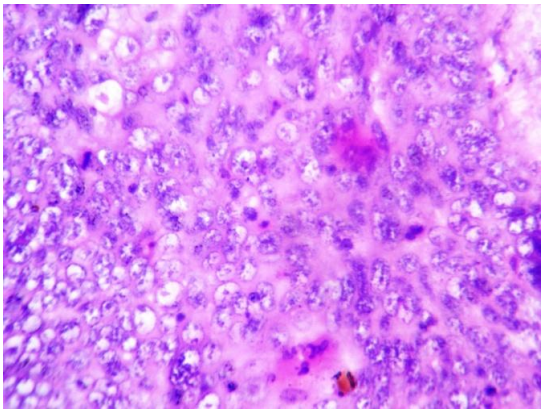


FIGURE 16: Squamous metaplasia (400X) HPE 6090/11

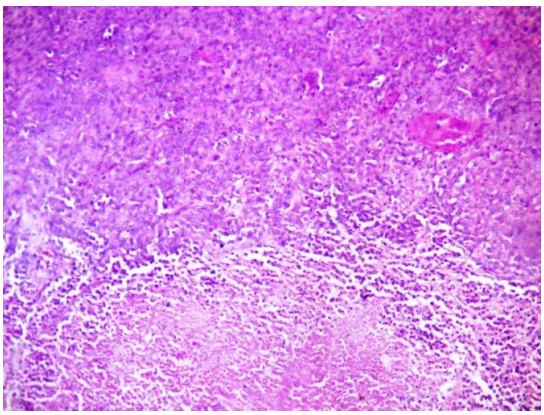


FIGURE 17: Necrosis (100X) HPE 6120/11

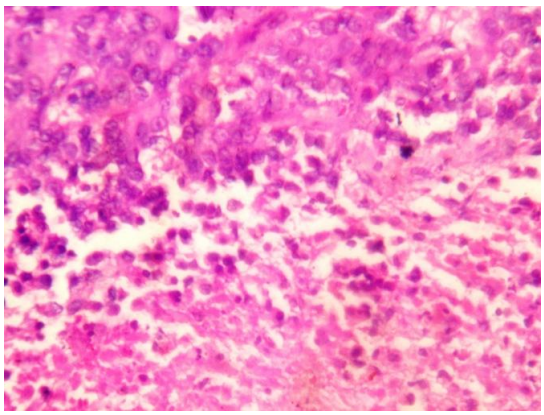


FIGURE 18: Necrosis (400X) HPE 6120/11

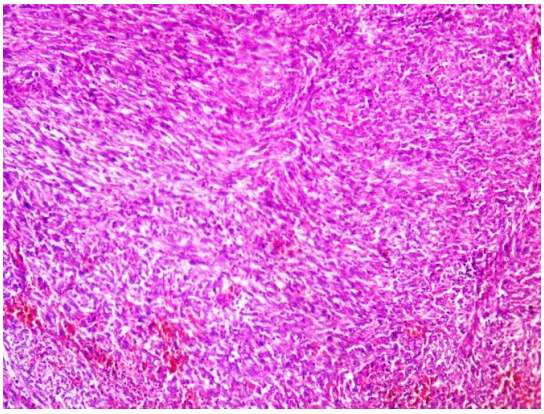


FIGURE 19: Sarcomatoid component (100X) HPE 5743/11

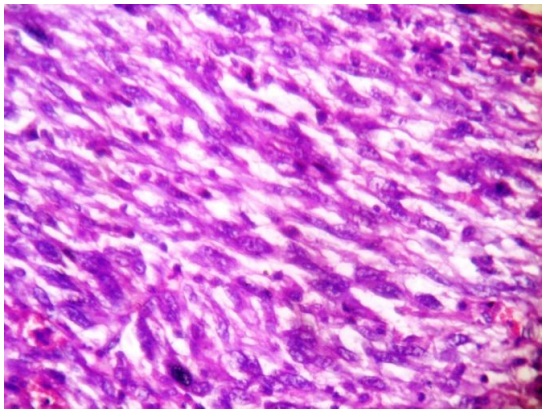


FIGURE 20: Sarcomatoid component (400X) HPE 5743/11

URINE CYTOLOGY POSITIVE IN TRANSITIONAL CELL CARCINOMAS

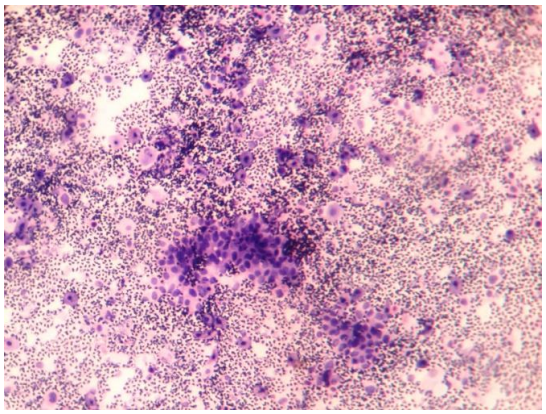


FIGURE 21: Urine cytology showing clusters of malignant epithelial cells (100X) C-3397/11

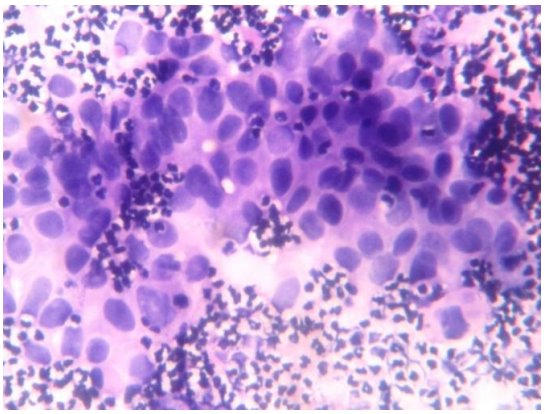


FIGURE 22: Malignant cells showing nuclear atypia and hyperchromatic nuclei (400X) C-3397/11

HIGH MICROVESSEL DENSITY

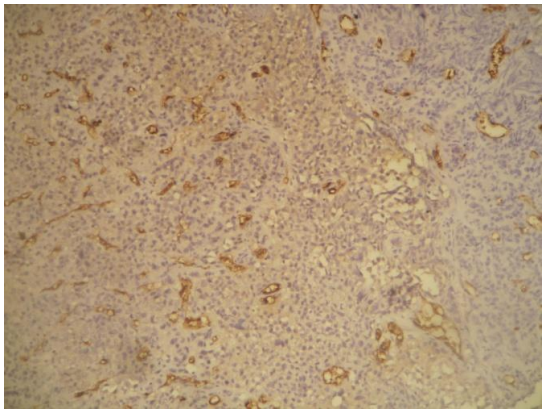


FIGURE 23: Transitional cell carcinoma of bladder- high grade showing high microvessel density- 102 vessels. (200X) HPE 6516/11

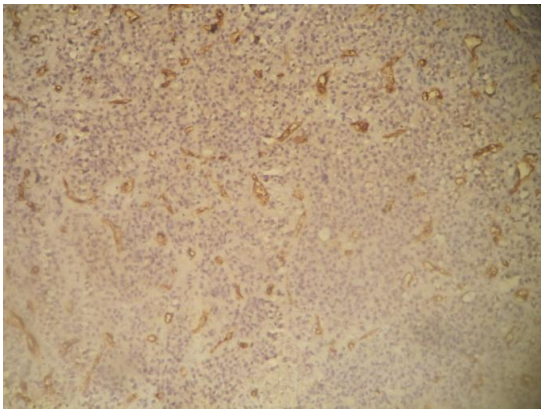


FIGURE 24: Transitional cell carcinoma of bladder- high grade showing high microvessel density- 95 vessels. (200X) HPE 165/11

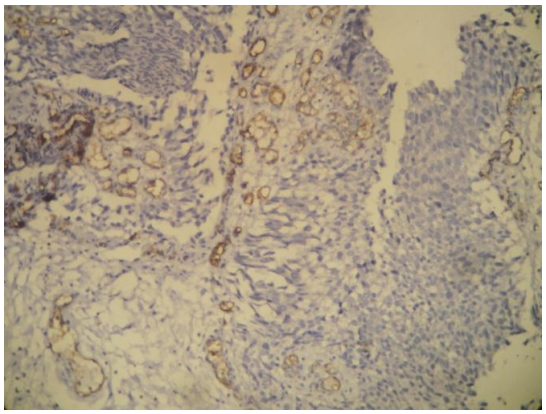


FIGURE 25: Transitional cell carcinoma of bladder- low grade showing high microvessel density- 51 vessels. (200X) HPE 1093/10

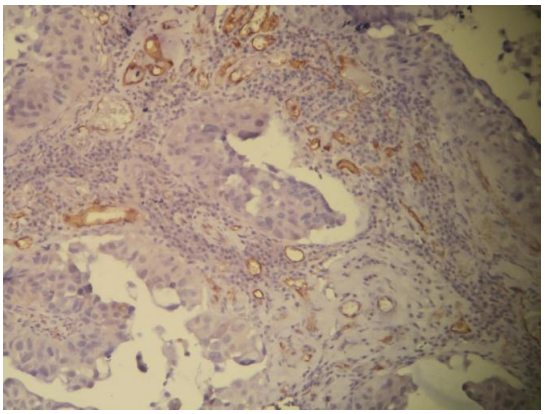


FIGURE 26: Transitional cell carcinoma of bladder- low grade showing high microvessel density- 48 vessels. (200X) HPE 6928/10

LOW MICROVESSEL DENSITY

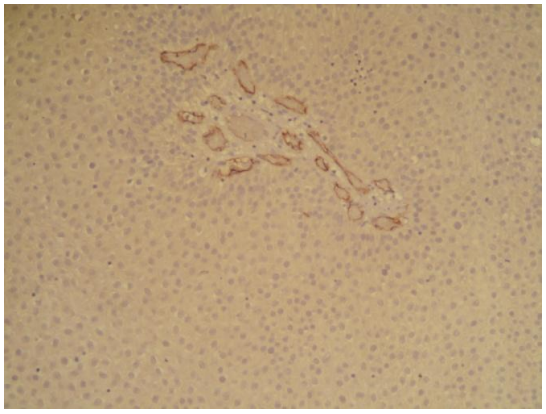


FIGURE 27: Transitional cell carcinoma of bladder- high grade showing low microvessel density- 15 vessels. (200X) HPE 1882/11

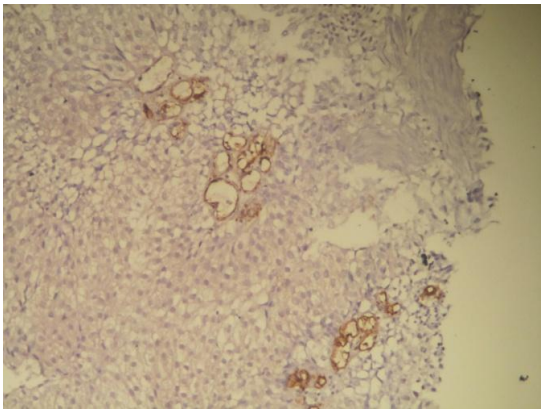


FIGURE 28: Transitional cell carcinoma of bladder- high grade showing low microvessel density-23 vessels. (200X) HPE 9467/10

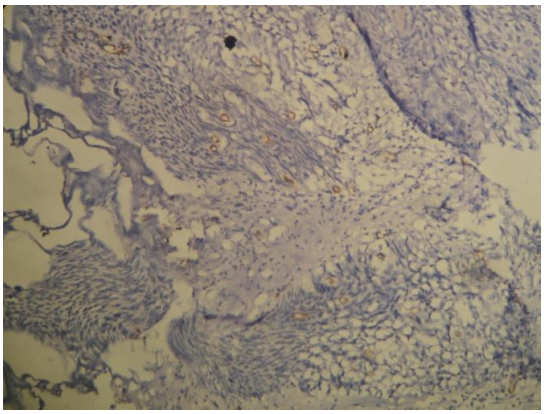


FIGURE 29: Transitional cell carcinoma of bladder- low grade showing low microvessel density- 22 vessels. (200X) HPE 10329/11

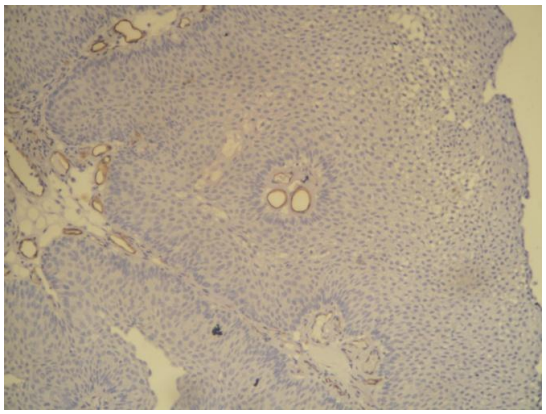


FIGURE 30: Transitional cell carcinoma of bladder- low grade showing low microvessel density- 21 vessels. (200X) HPE 10331/11

HER-2/neu NEGATIVE (0)

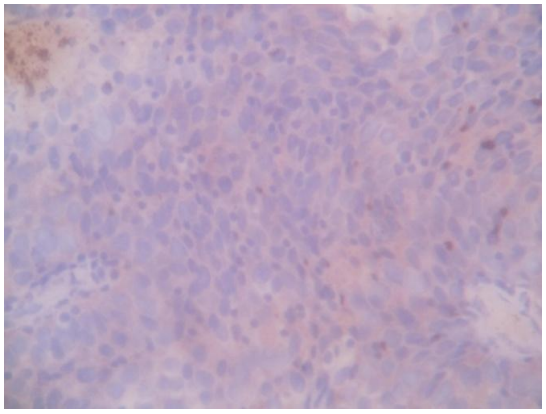


FIGURE 31: Transitional cell carcinoma of bladder- low grade showing no staining. (100X) HPE 8813/11

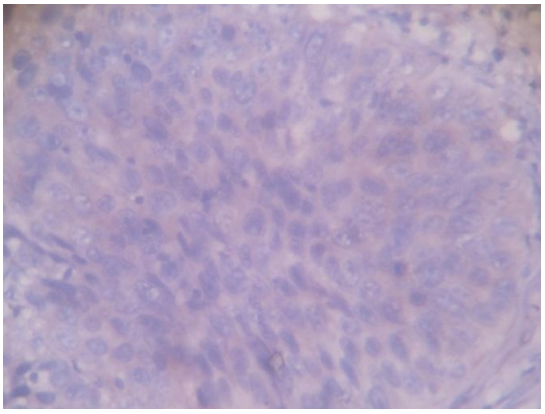


FIGURE 32: Transitional cell carcinoma of bladder- low grade showing no staining. (400X) HPE 8813/11

HER-2/neu NEGATIVE (1+)

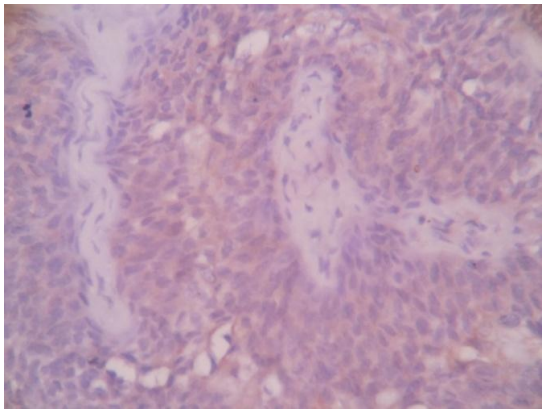


FIGURE 33: Transitional cell carcinoma of bladder- low grade showing weak staining in part of membrane of less than 10% of the cells staining. (100X) HPE 9719/11

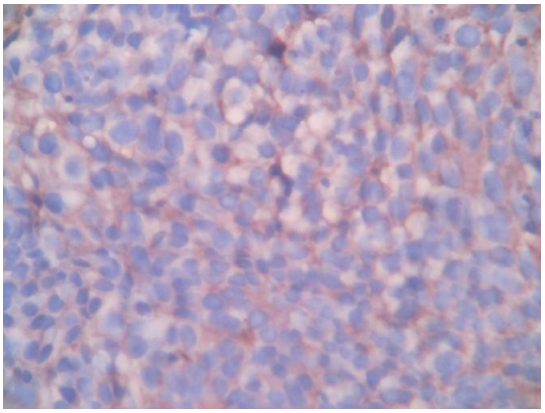


FIGURE 34: Transitional cell carcinoma of bladder- low grade showing weak staining in part of membrane of less than 10% of the cells staining. (400X) HPE 9719/11

HER-2/ neu POSITIVE (2+)

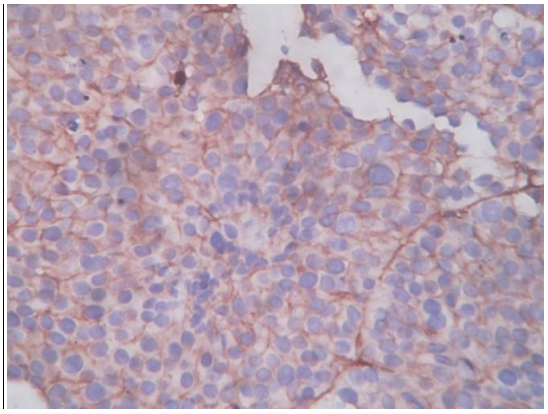


FIGURE 35: Transitional cell carcinoma of bladder- high grade showing complete membranous staining of moderate intensity in >10% of tumor cells. (100X) HPE 8596/10

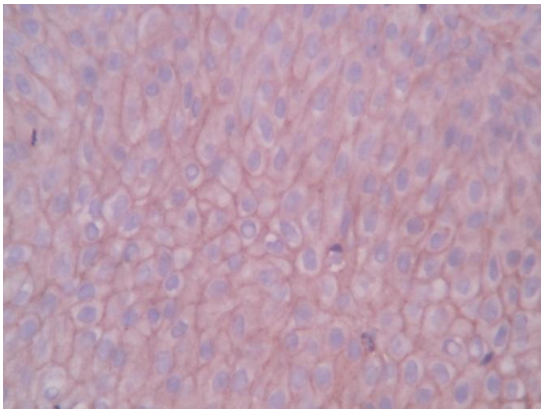


FIGURE 36: Transitional cell carcinoma of bladder- high grade showing complete membranous staining of moderate intensity in >10% of tumor cells. (400X) HPE 8596/10

HER-2/ neu POSITIVE (3+)

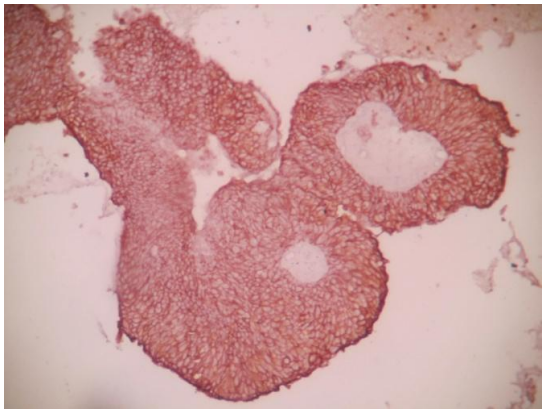


FIGURE 37: Transitional cell carcinoma of bladder- high grade showing strong complete membranous staining in more than 10 % of tumor cells. (100X) HPE 7219/11

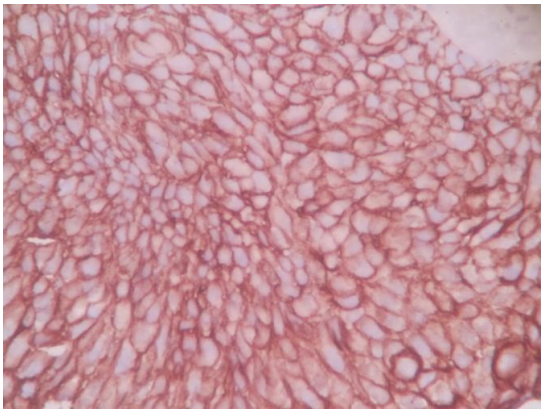


FIGURE 38: Transitional cell carcinoma of bladder- high grade showing strong complete membranous staining in more than 10 % of tumor cells. (400X) HPE 7219/11

DISCUSSION

Bladder cancer is the sixth most common malignancy in developed countries.[1,3] It ranks as the fourth and ninth most frequently diagnosed cancer in men and women, respectively, in the United States.[1] The incidence of bladder cancer in India is 3.2% in males and 0.7% in females.[87]

Though grade and stage are considered to be the best prognostic markers for bladder carcinoma, additional prognostic information is given by certain biologic markers. Microvessel density and HER-2/neu were considered as important markers which were correlated with other prognostic markers in various studies and give additional information.

In the present study, immunohistochemical evaluation was done in 50 cases of transitional cell carcinomas of bladder and an attempt was made to correlate the microvessel density and HER-2/neu expression with the known prognostic factors of bladder cancers and with recurrence.

Madras Medical College being a tertiary referral centre, about 0.57% of bladder carcinomas and 0.51% of TCCs were reported among the specimens received in the year 2010 and 2011. Among the bladder specimens received, 81.1% were malignant tumours.

The common tumour in bladder in this study was transitional cell carcinoma (88.6%) which is nearer to the study by Matalka et al[91] and Khaled El Gehani et al.[92] However, the percentage of squamous cell carcinoma and adenocarcinoma were more in this study (Table 41).

TABLE : 41 - COMPARISON OF DISTRIBUTION OF HISTOLOGICAL TYPES OF BLADDER CARCINOMA

Histological type	Matalka et al[93]	Khaled El Gehani et al[92]	Current study
Transitional cell carcinoma	95.7%	92.8%	88.6%
Adenocarcinoma	2.6%	2.4%	6.1%
Squamous cell carcinoma	1.7%	2.4%	5.3%
TCC and SCC	-	2.4%	-

This study showed that the highest incidence of transitional cell carcinomas occurred in 61 to 70 year age group. The age of transitional cell carcinoma patients ranged from patients 22 years to 82 years with the mean age of 58.74 years. This had a concurrence with the study done by Mohammad Reza Jalali Nadoushan et al[88] and Surendra B Kolla et al[89] (Table 42).

TABLE : 42 - COMPARISON OF MEAN AGE IN TRANSITIONAL CELL CARCINOMAS OF BLADDER

Studies	Mohammad Reza Jalali Nadoushan et al[88]	Surendra B Kolla et al[89]	Current study
Mean age(yrs)	56.3	58	58.74

The male:female ratio is 8:1 which was close to the study conducted by Matalka I et al[90] (9:1). The most common site of transitional cell carcinoma of bladder in this study was the right lateral wall (37.7%) followed by left lateral wall (29.8%). This was almost similar to the study by William T. Stephenson et al[93] who also showed the predominance of tumours arising from the lateral walls.

This study showed 6% of multiple tumours which was lower than the study by Igor Frank et al[94] and L. Santos et al[95] who showed the incidence of multiple tumours to be 14%and 12% respectively. In this study, the mean size of tumour was 4.1cm which showed concurrence with the study conducted by Igor Frank et al(4.1).[94]

In this study, papillary tumours were more common than the non-papillary(solid) tumours which was in concurrence with the study conducted by Thomas Quentin et al.[96] (Table 43)

TABLE : 43 - COMPARISON OF HISTOLOGICAL TYPE OF TRANSITIONAL CELL CARCINOMAS OF BLADDER

Histological type	Thomas Quentin et al[96]	Current study
Papillary	73.2%	82.2%
Non-papillary	26.8%	17.8%

In the present study, low grade tumours were more common. This was in concurrence with the study conducted by Matalka I et al.[91] (Table 44).

TABLE : 44 – COMPARISON OF GRADE OF TUMOUR

Grade	Matalka I et al[91]	Ali Canoglu et al[97]	Current study
Low grade	60%	44.1%	73.3%
High grade	40%	31.2%	26.7%
PUNLMP	-	24.7%	-

This study showed a higher proportion of T1 tumours followed by Ta tumours similar to the study by Eun Yong Choi et al.[98] However this did not concur with the studies by others who showed a predominance of tumours of other stage (Table 45).

TABLE : 45 - COMPARISON OF DEPTH OF TUMOUR

Depth of tumour	Matalka I et al[91]	Igor Frank et al[94]	Thomas Quentin et al[96]	Sari A et al[99]	Eun Yong Choi et al[98]	Current study
Ta	54.5%	1.9%	59.2%	13.7%	12.9%	29.7%
T1	17.3%	2.6%	5.6%	15.7%	50%	40.6%
T2	20%	31.3%	31%	21.6%	14.5%	25.7%
T3	7.3%	51.9%	4.2%	33.3%	11.3%	1%
T4	0.9%	12.3%	-	15.7%	11.3%	3%

This study showed most of the cases in stage 1 which is concurrent with the study conducted by Khaled El Gehani et al[88] and E.A. Philp et al[95](Table 46).

TABLE : 46 - COMPARISON OF STAGE OF INFILTRATIVE TUMOURS

Stage	Khaled El Gehani et al[92]	E.A. Philp et al[99]	Current study
1	71.4%	59.3%	57.7%
2	26.2%	14.2%	36.6%
3	2.4%	15%	5.7%
4	-	11.5%	-

This study showed a recurrence of 29.7% which was concurrent with the study done by Matalka I et al[91] in Jordan, Asia. However, studies by Rawaa G Al-Tereihi et al[104] which was done in Iraq, Asia

showed increased recurrence rate of 58.33% (Table 47). This study showed that many of the recurrent tumours presented with lower stage. Hence other prognostic factors like microvessel density and HER-2/neu expression assumed clinical significance.

TABLE : 47 - COMPARISON OF RECURRENCE OF TUMOUR

Recurrence	Rawaa G Al-Tereihi et al[104]	Matalka I et al[91]	Current study
Present	58.33%	31.4%	29.7%
Absent	41.67%	68.6%	70.3%

TABLE : 48 - COMPARISON OF MEAN MICROVESSEL DENSITY WITH WORLD STATISTICS

Studies	Grade		Infiltration		Stage			Recurrence/ progression	
	Low	High	Present	Absent	1	2	3	Present	Absent
Eun Yong Choi et al[98]	94.5	109.5	-	-	-	-	-	131.1	94.5
Khaled El Gehani et al[92]	65.8	106.3	-	-	72.1	104.8	86.6	-	-
N.E.Stavropoulos et al[101]	20.8	16.4	20.73	17.58	-	-	-	19.61	19.1
E.A. philp et al[100]	47.7	51.8	-	-	46.19	53.90	52.2	-	-
C.K.Hawke et al[105]	77.4	90.8	-	-	-	-	-	-	-
Jonathan C. Goddard et al[106]	75.8	81.3	65.6	85.5	-	-	-	-	-
AliCanoglu et al[97]	90.4	95.7	88.9	94.0	-	-	-	92.7	87.4
Current study	35.0	38.6	44.8	34.9	36.9	32.4	30.0	39.9	33.9

CORRELATION OF MICROVESSEL DENSITY WITH KNOWN CLINICO-PATHOLOGICAL PROGNOSTIC FACTORS

E.A. Philp et al (1996) studied 113 cases of transitional cell carcinoma of bladder and demonstrated statistically significant association between MVD and tumour stage and with prognosis.[100]

C.K.Hawke et al (1998) studied 42 cases of transitional cell carcinomas and found a statistically significant association between MVD and survival of patients. Though the mean MVD was increased with increasing grade and stage, the correlation was not statistically significant.[105]

Eun Yong Choi et al (1999) studied 67 cases of transitional cell carcinomas of bladder and found no correlation between MVD with stage or grade of the tumour. However they found a significant association with tumour progression.[98]

L. Santos et al (2003) studied 66 superficial papillary carcinomas and found that MVD was an independent prognostic factor for recurrence.[95]

Jonathan C. Goddard et al (2003) studied 180 cases of TCCs and found a statistically significant correlation between MVD with stage and

progression of the tumour. Though the mean MVD was increased in high grade tumours, the association was not statistically significant.[106]

AliCanoglu et al (2004) studied 43 superficial TCCs and 34 invasive TCCs and found statistically significant correlation between MVD and stage, grade, infiltration and tumour progression and recurrence.[97]

N.E.Stavropoulos et al (2004) studied 127 cases of superficial bladder carcinomas and showed that there was no significant association of MVD with stage, grade and recurrence.[101]

Khaled El Gehani et al (2005) studied 42 cases of bladder carcinomas and found statistically significant association of MVD with tumour grade and stage and it was not affected by age and sex of the patients.

In comparison with the above studies, this study showed an increase in MVD in high grade and in recurrent tumours, but this association was not found to be statistically significant. This study showed decreasing MVD with stage of the tumour, with infiltration and with size which was statistically insignificant. The MVD values were also increased in multiple tumours, tumours with sarcomatoid component and in tumours with positive urine cytology. But these values were also

statistically insignificant. MVD values showed statistically significant increase in male gender and in non-papillary (solid) tumours.

Though this study showed a decrease in mean MVD with increasing stage of the tumour, it can be seen that patients presenting with high MVD (>50) were presenting with recurrence even when they present in lower stage and HER-2/neu negative. Hence, more patients presenting with early stage need to be evaluated for MVD and closely followed up for possible recurrence.

One case with a maximum MVD of 102 was an early stage Ta tumour presenting for the first time showed positive HER-2/neu status. Since the MVD values were high, a close follow-up of this patient for recurrence could assume greater clinical significance.

TABLE : 49 - COMPARISON OF HER-2/NEU EXPRESSION WITH WORLD STATISTICS

Studies	HER-2/neu positive	HER-2/neu negative
Mohammad Reza Jalali Nadoushan et al[88]	37.3%	62.7%
Donna E. Hansel et al[102]	36%	64%
Surendra B. Kolla et al[89]	55.6%	44.4%
Aurora alexa et al[103]	35.6%	64.4%
Rawaa G Al-Tereihi et al[104]	41.6%	58.4%
Khaled El Gehani et al[92]	59%	41%
Current study	44%	56%

CORRELATION OF HER-2/NEU EXPRESSION WITH KNOWN CLINICO-PATHOLOGICAL PROGNOSTIC FACTORS

Mohammad Reza Jalali Nadoushan et al (2007) assessed 75 cases of transitional cell carcinomas of bladder and found a definitive association between HER-2/neu expression and grade of the tumour.(88)

Donna E. Hansel et al (2008) examined 53 invasive high-grade urothelial carcinomas and demonstrated that HER-2/neu targeted therapy can be useful for metastatic lesions.(102)

Surendra B. Kolla et al (2008) examined 90 cases of invasive transitional cell carcinoma of the urinary bladder and demonstrated that HER-2/neu expression is associated with advanced bladder cancer and influences disease progression in these patients.(89)

Aurora alexa et al (2010) studied the expression of HER-2/neu in 59 cases of urothelial carcinomas and demonstrated statistically significant correlation with tumour grade. However, HER-2/neu expression was not correlated with tumour stage.(103)

Rawaa G Al-Tereihi et al (2011) studied 60 cases of bladder carcinoma and demonstrated statistically significant association between HER-2/neu expression with grade and stage of bladder carcinoma.

However the association between HER-2/neu expression and recurrence was not statistically significant.[104]

Khaled El Gehani et al (2011) studied 39 cases of bladder carcinoma and demonstrated statistically significant correlation between HER-2/neu expression with tumour stage. However, though the expression of HER-2/neu was more frequent in high-grade than in low-grade carcinomas, but the difference was not statistically significant. Patients' age and sex were not related to HER-2 over expression.[92]

In comparison with the above studies, this study showed a statistically significant association between HER-2/neu expression and grade of the tumour. This study also showed a statistically significant association between HER-2/neu and urine cytology positivity. Though this study showed increased HER-2/neu expression in older age group, male gender, multiple tumours, increasing size and stage, necrosis, sarcomatoid component and recurrence, statistically significant association could not be ascertained. This study also showed an increased HER-2/neu expression in papillary tumours when compared to non-papillary tumours which was not statistically significant.

Among the 7 cases of recurrence with high MVD (>50), 5 cases were negative for HER-2/neu expression. More studies are required to assess if there is any inhibitory effect of HER-2/neu over-expression on microvessel density.

SUMMARY

- The percentage of bladder carcinomas among the 19898 surgical samples received at Madras Medical College in the year 2010 and 2011 is 5.7%.
- The distribution of non-neoplastic bladder lesions was 20.9% and malignant tumours were 81.1%.
- Transitional cell carcinomas were the most common carcinoma of bladder constituting 88.6% of cases.
- Transitional cell carcinomas had a peak incidence in the age group of 61 – 70 years. The mean age of presentation is 58.74 yrs.
- 78.2% of transitional cell carcinomas occur in males and 21.8% in females.
- The most common location of transitional cell carcinoma was at the right lateral wall which constituted about 37.7% of the cases.
- 94% of transitional cell carcinomas were single and 6% were multiple.
- The mean size of transitional cell carcinomas was 4.1 cm.
- Papillary tumours constituted 82.2% of cases and solid tumours, 17.8% of cases.

- Low grade urothelial carcinomas were more common accounting for 73.3% of cases.
- 70.3% of tumours were infiltrative and 29.7% were superficial tumours.
- 40.6% tumours presented in T1 stage (invasion upto lamina propria).
- Most of the tumours presented in stage 1(40.6%).
- Squamous metaplasia was present in 14.9% of cases, necrosis in 18.8% of cases and sarcomatoid component in 3% of cases.
- Urine cytology was positive in 58.3% of cases.
- Recurrence was present in 29.7% of cases.
- The mean microvessel density was 36.44.
- The mean MVD values showed statistically significant increase in case of male gender, non-papillary tumours and in TCCs with necrosis.
- The mean MVD values showed an increase in multiple tumours, high grade, in tumours with sarcomatoid component, positive urine cytology and in recurrent tumours. However they were statistically insignificant.

- The mean MVD values showed a decrease with increasing stage, size and infiltration and this association was statistically insignificant.
- Though MVD values were reducing with stage, patients with high microvessel density (>50) presented with recurrence.
- HER-2/neu expression was seen in 44% of cases.
- Statistically significant association was found between HER-2/neu expression and grade and urine cytology positive tumours.
- HER-2/neu expression was increased in older patients, males, multiple tumours, tumours with increased size, stage, infiltration, necrosis, sarcomatoid component, necrosis and in recurrent tumours but these were statistically insignificant.
- HER-2/neu expression was increased in papillary tumours than non-papillary tumours, but this had no statistical significance.
- The mean MVD values were increased in HER-2/neu positive tumours but this was not statistically significant.

CONCLUSION

The incidence of bladder carcinoma was lower in this study group than the western population. Many patients presented in older age with a mean age of 58.74 with predominance in males. However, there was an increase in squamous cell carcinomas and adenocarcinomas in the study. This study also showed a reduced rate of recurrence than the western population. The mean microvessel density was found to be 36.44 and HER-2/neu expression was seen in 44% of the cases.

An increased HER-2/neu expression was noted in older patients, male gender, multiple tumours, recurrent tumours, increasing size, stage, infiltration and in the presence of necrosis and sarcomatous component, but was not statistically significant. HER-2/neu showed an increased expression in papillary tumours with no statistical significance. Both microvessel density and HER-2/neu expression were increased in cases of recurrent tumours which was not statistically significant. There is no statistically significant difference in microvessel density of HER-2/neu positive and negative tumours.

Microvessel density had a statistically significant association with necrosis, non-papillary tumours and in male gender. HER-2/neu expression showed statiscally significant association with grade and urine

cytology positivity. An increased microvessel density was noted in multiple, high grade, recurrent, positive urine cytology tumours and in tumours with sarcomatous component which was statistically insignificant. The values were reduced with increasing stage, size and infiltration which was not found to be statistically significant.

In this study, though MVD was found to decrease with stage, the patients with high MVD values i.e >50 were presenting with recurrence. Also many of the recurrent tumours presented in early stage. Hence more patients presenting with early stage tumour for the first time need to be evaluated for MVD and closely followed up for possible recurrence. Thus a larger sample size will help in identifying the association between the stage, MVD status and recurrence to formulate treatment strategies and possible targeted therapy.

MASTER CHART

S. NO	HPE	AGE	SEX	PROC	SIZE	SITE	DIAG	PAP	GR	INFIL	SQ META	NEC	SACOM	REC	T	STAGE	MVD	HER-2/NEU	CYTO	MALIG
1	4868/11	35	F	TURBT	4	Dome	TCC	Y	Low	P	A	A	A	A	T1	I	28	1	C-1693/11	Neg
2	4846/11	80	M	TURBT	3	Rt lat wall	TCC	Y	Low	P	A	A	A	A	T2A	II	48	2		
3	4963/11	55	M	TURBT	6	Rt lat wall	TCC	Y	Low	P	A	A	A	P-2008	T1	I	38	2		
4	5743/11	62	M	TURBT	4	Multiple	TCC	Y	High	P	A	A	P	P-6mon	T2A	II	57	2	C-2157/11	Pos
5	5746/11	78	M	TURBT	5	Rt lat wall	TCC	Y	Low	P	A	A	A	A	T2A	II	29	3		
6	6090/11	67	M	TURBT	4	Lt lat wall	TCC	Y	High	P	P	P	A	A	T1	I	8	2		
7	6120/11	43	M	TURBT	7	Entire	TCC	Y	High	P	P	P	A	A	T1	I	32	1		
8	5211/11	60	M	TURBT	5	Pos wall	TCC	Y	Low	P	A	A	A	A	T1	I	37	1	C-1901/11	Pos
9	4762/11	60	M	TURBT	4	Lt lat wall	TCC	Y	High	P	A	P	A	P-2mon	T1	I	35	3		
10	5541/11	65	M	TURBT	3	Multiple	TCC	Y	High	P	A	A	A	P-8mon	T1	I	43	3		
11	6516/11	67	M	TURBT	7	Entire	TCC	Y	High	A	A	A	A	A	TA	0	102	2	C-2339/11	Pos
12	7149/11	55	M	Rad cyst	8	Entire	TCC	Y	High	P	A	P	A	A	T4A	III	22	3	C-2316/11	Pos
13	7933/11	51	M	Rad cyst	5	Rt lat wall	TCC	Y	Low	P	A	A	A	A	T2A	II	23	2		
14	6620/11	65	M	TURBT	3	Rt lat wall	TCC	Y	High	P	P	A	A	A	T1	I	26	0		
15	7770/11	56	M	TURBT	1.5	Lt lat wall	TCC	Y	Low	A	A	A	A	P-2008	TA	0	37	1		
16	7559/11	68	M	TURBT	6	Rt lat wall	TCC	Y	High	P	P	A	A	P-1mon	T2A	II	43	1		
17	2341/11	60	M	TURBT	6	Rt lat wall	TCC	Y	High	P	A	A	A	P-12mon	T1	I	55	1		
18	1882/11	45	F	TURBT	0.5	Dome	TCC	Y	High	P	A	A	A	A	T1	I	15	0		
19	3258/11	59	M	TURBT	3	Base	TCC	Y	Low	A	A	A	A	P-2mon	TA	0	53	1		
20	3311/11	81	M	TURBT	2	Multiple	TCC	Y	Low	A	A	A	A	A	TA	0	48	1		
21	3738/11	75	M	TURBT	3	Multiple	TCC	Y	Low	P	P	A	A	A	T2A	II	28	1		
22	13/11	65	M	TURBT	4	Base	TCC	Y	High	P	A	A	A	P-3mon	T1	I	65	0		
23	71/11	54	M	Small bx	5	Dome	TCC	N	High	P	A	A	A	P-1mon	T1	I	36	0		

24	1151/11	47	F	TURBT	2	Lt lat wall	TCC	Y	Low	P	A	A	A	A	T1	I	35	1		
25	1510/11	61	M	TURBT	3	Lt lat wall	TCC	Y	Low	P	A	A	A	P-3mon	T1	I	55	1		
26	8736/11	59	M	TURBT	3	Rt lat wall	TCC	Y	Low	A	A	A	A	P-2mon	TA	0	32	0		
27	8813/11	46	M	TURBT	6	Base	TCC	Y	Low	P	A	A	A	A	T1	I	18	0	C-3366/11	Neg
28	8922/11	65	M	TURBT	7	Entire	TCC	Y	Low	P	A	A	A	A	T2A	II	35	3	C-3397/11	Pos
29	9538/11	65	F	TURBT	5	Base	TCC	Y	Low	P	A	A	A	A	T2A	II	29	1	C-3662/11	Neg
30	9719/11	57	M	TURBT	5	Lt lat wall	TCC	Y	Low	P	A	A	A	A	T1	I	32	1		
31	10074/11	68	M	TURBT	8	Lt lat wall	TCC	Y	Low	P	A	A	A	A	T1	I	32	1		
32	10329/11	51	M	TURBT	3	Rt lat wall	TCC	Y	Low	A	A	A	A	P-1mon	TA	0	22	1		
33	10331/11	57	M	TURBT	4	Lt lat wall	TCC	y	Low	P	A	A	A	P-2mon	T2A	II	21	2		
34	6241/11	65	M	TURBT	6	Entire	TCC	y	Low	P	P	A	A	A	T1	I	31	3	C-2285/11	Pos
35	7219/11	65	M	TURBT	5	Lt lat wall	TCC	Y	High	P	A	A	A	A	T2A	II	30	3	C-2562/11	Pos
36	165/11	50	M	TURBT	4	Lt lat wall	TCC	N	High	P	A	A	A	P-3mon	T1	I	95	3		
37	1580/10	70	M	TURBT	3	Multiple	TCC	Y	High	P	A	A	A	A	T1	I	35	3		
38	1096/10	55	M	TURBT	2	Lt lat wall	TCC	Y	Low	P	A	A	A	A	T1	I	30	2		
39	6928/10	75	M	TURBT	3	Lt lat wall	TCC	Y	Low	P	P	P	A	A	T2A	II	48	1		
40	965/10	58	M	TURBT	4	Lt lat wall	TCC	Y	Low	P	A	A	A	A	T1	I	30	1		
41	1048/10	45	F	TURBT	4	Rt lat wall	TCC	N	High	P	A	P	A	P-2009	T2A	II	32	2		
42	1093/10	47	M	TURBT	3	Dome	TCC	N	Low	P	A	A	A	A	T1	I	51	1		
43	1380/10	68	F	Simp cyst	6	Dome	TCC	Y	High	P	A	A	A	P-2009	T2A	II	23	2		
44	8725/10	65	M	TURBT	2	Rt lat wall	TCC	N	Low	P	A	A	A	A	T3B	III	38	1	C-2946/10	Pos
45	7741/10	45	F	Rad cyst	7	Entire	TCC	Y	Low	P	A	A	A	P-1mon	T2B	II	23	1		
46	3880/10	70	M	TURBT	3	Rt lat wall	TCC	Y	Low	A	A	A	A	A	TA	0	38	1		
47	8596/10	64	M	Small bx	4	Rt lat wall	TCC	Y	Low	A	A	A	A	A	TA	0	26	2		
48	9467/10	49	M	TURBT	6	Rt lat wall	TCC	Y	High	P	P	P	A	A	T2A	II	23	1		
49	6759/10	75	M	TURBT	3	Rt lat wall	TCC	Y	High	P	A	P	A	P-2009	T2A	II	28	3	C-2335/10	Neg
50	5061/10	66	F	TURBT	6	Rt lat wall	TCC	Y	High	P	P	A	A	A	T2A	II	22	2		
51	2351/11	60	M	TURBT	5	Pos wall	TCC	N	Low	P	A	A	A	A	T1	I				

52	2353/11	58	M	TURBT	4	Lt lat wall	TCC	Y	Low	P	A	A	A	P-2010	T1	I				
53	2625/11	80	M	TURBT	2	Rt lat wall	TCC	Y	Low	P	A	A	A	A	T1	I				
54	2907/11	54	M	TURBT	2	Rt lat wall	TCC	Y	Low	A	A	A	A	A	TA	0				
55	4719/11	35	M	Small bx	5	Rt lat wall	TCC	Y	Low	A	P	P	A	A	TA	0				
56	4842/11	55	F	TURBT	3	Pos wall	TCC	Y	Low	A	A	A	A	P-2009	TA	0				
57	3943/11	62	F	TURBT	4	Rt lat wall	TCC	Y	Low	P	A	A	A	A	T1	I				
58	4527/11	64	F	TURBT	3	Pos wall	TCC	Y	Low	A	A	A	A	A	TA	0				
59	158/10	65	F	TURBT	3	Rt lat wall	TCC	Y	Low	P	A	A	A	A	T1	I			C-3308/09	Pos
60	2446/10	52	M	TURBT	4	Lt lat wall	TCC	Y	Low	P	P	A	A	A	T1	I				
61	2552/10	70	M	TURBT	3	Multiple	TCC	Y	Low	A	A	P	A	A	TA	0			C-899/12	Neg
62	2808/10	45	F	TURBT	4	Rt lat wall	TCC	Y	Low	A	A	A	A	A	TA	0			C-997/10	Pos
63	2825/10	45	M	Rad cyst	7	Entire	TCC	N	Low	P	A	A	A	P-2mon	T2A	II				
64	2863/10	65	M	TURBT	3	Lt lat wall	TCC	Y	Low	A	A	A	A	A	TA	0			C-1018/10	Neg
65	3106/10	22	M	TURBT	4	Lt lat wall	TCC	Y	High	P	A	A	A	A	T1	I				
66	3434/10	47	M	TURBT	3	Base	TCC	Y	Low	A	A	P	A	A	TA	0				
67	3547/10	60	M	TURBT	4	Rt lat wall	TCC	N	High	P	A	P	A	A	T2A	II				
68	3673/10	65	F	Small bx	2	Dome	TCC	Y	Low	A	A	A	A	A	TA	0				
69	3971/10	65	M	Small bx	4	Rt lat wall	TCC	N	Low	P	A	A	A	A	T1	I			C-1317/10	Pos
70	4035/10	73	F	TURBT	6	Entire	TCC	Y	Low	P	A	A	A	A	T1	I				
71	4155/10	33	M	TURBT	0.5	Rt lat wall	TCC	N	Low	A	A	P	A	A	TA	0				
72	4288/10	65	F	TURBT	6	Rt lat wall	TCC	N	Low	A	A	A	A	A	TA	0				
73	4374/10	51	M	TURBT	3	Base	TCC	Y	Low	P	A	A	A	A	T1	I				
74	4455/10	62	M	Rad cyst	4	Rt lat wall	TCC	Y	High	P	A	A	A	P-2009	T2B	II				
75	4687/10	78	M	TURBT	3	Lt lat wall	TCC	N	Low	A	A	A	A	A	TA	0				
76	5038/10	47	F	Rad cyst	3.5	Rt lat wall	TCC	N	Low	P	A	P	A	P-2mon	T2A	II				
77	5336/10	82	M	TURBT	4	Lt lat wall	TCC	Y	Low	P	A	A	A	A	TI	I			C-1741/10	Pos
78	3571/10	46	F	TURBT	4	Rt lat wall	TCC	N	High	P	P	P	A	A	T1	I				
79	6267/10	34	M	Rad cyst	5	Lt lat wall	TCC	Y	Low	P	A	A	A	A	T1	I				

80	6439/10	65	F	TURBT	3	Dome	TCC	Y	Low	A	A	A	A	A	TA	0				
81	6716/10	75	M	TURBT	3	Lt lat wall	TCC	Y	Low	A	A	A	A	A	TA	0				
82	7534/10	49	M	Rad cyst	6	Rt lat wall	TCC	Y	Low	P	P	P	A	P-1mon	T2A	II				
83	7838/10	67	M	Small bx	5	Rt lat wall	TCC	Y	Low	A	A	A	P	A	TA	0			C-2680/10	Pos
84	9132/10	60	M	TURBT	3	Lt lat wall	TCC	Y	Low	A	A	A	A	A	TA	0				
85	348/11	56	M	Small bx	4	Rt lat wall	TCC	Y	Low	A	A	A	A	A	TA	0				
86	474/11	60	F	TURBT	2.5	Rt lat wall	TCC	Y	Low	P	A	A	A	P-4 mon	T1	I			C-105/11	Neg
87	567/11	72	M	TURBT	2	Rt lat wall	TCC	Y	Low	P	A	A	A	P-2004	T1	I			C-168/11	Neg
88	610/11	40	F	TURBT	4	Lt lat wall	TCC	N	Low	P	A	P	A	A	T2A	II				
89	1399/11	75	M	TURBT	5	Rt lat wall	TCC	N	Low	P	A	P	A	A	T2A	II			C-463/11	Pos
90	1614/11	79	M	TURBT	4.5	Lt lat wall	TCC	N	High	P	A	A	A	A	T1	I				
91	1036/11	45	M	TURBT	2	Lt lat wall	TCC	Y	Low	A	A	A	A	A	TA	0				
92	7769/11	60	M	TURBT	2	Rt lat wall	TCC	Y	Low	A	A	A	A	A	TA	0			C-2897/11	Neg
93	8175/11	31	M	TURBT	1	Lt lat wall	TCC	Y	Low	A	A	A	A	A	TA	0				
94	8473/11	80	M	TURBT	5	Rt lat wall	TCC	Y	Low	A	P	A	A	P-1 mon	TA	0				
95	8804/11	58	M	TURBT	6	Lt lat wall	TCC	Y	Low	P	A	P	A	P-3yrs	T1	I				
96	8910/11	32	M	TURBT	3	Lt lat wall	TCC	Y	Low	A	A	A	A	A	TA	0				
97	9007/11	52	M	TURBT	4	Lt lat wall	TCC	Y	Low	P	A	A	A	P-1yr	T1	I			C-3338/11	Neg
98	9838/11	65	M	Small bx	7	Entire	TCC	Y	Low	P	A	A	A	A	T4A	III				
99	9841/11	65	M	Small bx	6	Lt lat wall	TCC	Y	Low	P	A	A	A	A	T4A	III				
100	5932/11	42	F	TURBT	4	Rt lat wall	TCC	Y	Low	P	P	A	A	A	T2A	II				
101	5999/11	51	M	Rad cyst	10	Entire	Sa Ca	N	_	P	_	_	_	A	T2B	II				

KEY TO MASTER CHART

Proc	-	Procedure done
Pap	-	Papillary pattern
Infil	-	Infiltration
Sq meta	-	Squamous metaplasia
Nec	-	Necrosis
Sa com	-	Sarcomatoid component
Rec	-	Recurrence
MVD	-	Miicrovessel density
Malig	-	Malignancy
M	-	Male
F	-	Female
TURBT	-	Transurethral resection of bladder tumour
Rad cyst	-	Radical cystectomy
Small bx	-	Small biopsy
Simple cyst	-	Simple cystectomy
Rt lat wall	-	Right lateral wall
Lt lat wall	-	Left lateral wall
Pos wall	-	Posterior wall
TCC	-	Transitional cell carcinoma
Y	-	Yes
N	-	No
P	-	Present
A	-	Absent
Pos	-	Positive
Neg	-	Negative

BIBLIOGRAPHY

1. Jemal A, Siegel R, Ward E et al. Cancer statistics, 2007. CA Cancer J Clin 2006; 57; 43–66.

2. Charles Jameson. The pathology of bladder cancer. Cambridge University Press 978-0-521-88456-3.

3. Kirkali Z, Chan T, Manoharan M et al. Bladder cancer: epidemiology, staging and grading, and diagnosis. Urology 2005; 66, 4–34.

4. J.C. Goddard. The use of microvessel density in assessing human urological tumours. BJU International (2001), 87, 866±875.

5. Petros D. Grivas. Urothelial carcinomas: a focus on human epidermal receptors signalling. Am J Transl Res 2011;3(4):362-373.

6. Kenneth Wester. HER-2 - A Possible Target for Therapy of Metastatic Urinary Bladder Carcinoma. Acta Oncologica 41 (2002).

7. Lopez-Beltran A et al. Non-invasive urothelial neoplasms: according to the most recent WHO classification. Eur Urol 2004; 46:170-6.

8. Lopez-Beltran A, Sauter G, Gasser T et al. Urothelial tumors: infiltrating urothelial carcinoma. In: Eble JN, Sauter G, Epstein JI, Sesterhenn I, eds. World Health Organization Classification of Tumors: Pathology and Genetics of Tumors of the Urinary System and Male Genital Organs. Lyon: IARC Press, 2004.

9. Cheng L, Pan CX, Yang XJ et al. Small cell carcinoma of the urinary bladder: a clinicopathologic analysis of 64 patients. Cancer 2004; 101: 957–62.

10. McDonald DF, Lund RR. The role of the urine in vesical neoplasms: I. Experimental confirmation of the urogenous theory of pathogenesis. J Urol. 1954;71:560.

11. Miller AB. The etiology of bladder cancer from the epidemiological viewpoint. Cancer Res. 1977;37:2939.

12. Miller AB, Morrison B. Artificial sweeteners and human bladder cancer. Lancet 1977;2:578.

13. Worth PHL. Cyclophosphamide and the bladder. Br Med J. 1971;3:182.

14. Dale GA, Smith RB. Transitional cell carcinoma of the bladder associated with cyclophosphamide. J Urol. 1974;112:603.

15. Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB (2003). Cancer Incidence in Five Continents. IARC Scientific Publications No155. IARC Press: Lyon.

16. Mostofi FK, Sobin LH, Torloni H. Histological typing of urinary bladder tumours. Geneva: World Health Organization, 1973.

17. Pauwels RPE, Schapers RFM, Smeets AWGB, et al. Grading in superfi cial bladder cancer: morphological criteria. Br J Urol 1988; 61: 129–134.

18. Schapers RF, Pauwels RP, Wijnen JT, et al. A simplified grading method of transitional cell carcinoma of the urinary bladder: reproducibility, clinical significance and comparison with other prognostic parameters. Br J Urol 1994; 73: 625–631. 162.

19. Bostwick DG, Mikuz G. Urothelial papillary (exophytic) neoplasms. Virchows Arch 2002; 441: 109–116.

20. Mostofi FK, Davis CJ, Sesterhenn IA. WHO histologic typing of urinary bladder tumors. Berlin: Springer, 1999.

21. Cheng L, Neumann RM et al: Tumor size predicts the survival of patients with pathologic stage T2 bladder carcinoma: A critical evaluation of the depth of muscle invasion. Cancer 1999; 85:2638-2647.

22. Pagano F GA, Milani C, B assi P et al. Prognosis of baldder cancer I. Risk factors in superficial transitional cell carcinoma. Eur Urol 1987; 13: 145–9.

23. Skinner DG: Current perspectives in the management of high-grade invasive bladder cancer. Cancer 1980; 45:1866-1874.

24. Jordan AM, Weingarten J, Murphy WM: Transitional cell neoplasms of the urinary bladder. Can biologic potential be predicted from histologic grading? Cancer 1987; 60:2766-2774.

25. Heney NM, Ahmed S, Flanagan MJ et al. National Bladder Cancer Collaborative Group A: Superficial bladder cancer. Progression and recurrence. J Urol 1983; 130:1083-1086

26. Stephenson WT, Holmes FF, Noble MJ, Gerald KB: Analysis of bladder carcinoma by subsite. Cystoscopic location may have prognostic value. Cancer 1990; 66:1630-1635.

27. Afonso J, Santos LL, Amaro T, Lobo F, Longatto-Filho A: The aggressiveness of urothelial carcinoma depends to a large extent on

lymphovascular invasion – the prognostic contribution of related molecular markers. Histopathology 2009; 55:514-524.

28. Bochner BH, Cote RJ, Weidner N, Groshen S, Chen S-C, Skinner DG, Nichols PW: Angiogenesis in bladder cancer. Relationship between microvessel density and tumor prognosis. J Natl Cancer Inst 1995; 87:1603-1612.

29. Esrig D, Spruck CH, Nichols PW, Chaiwun B, Steven K, Groshen S, Chen SC, Skinner DG, Jones PA, Cote RJ: p53 nuclear protein accumulation correlates with mutations in the p53 gene, tumor grade, and stage in bladder cancer. Am J Pathol 1993; 143:1389-1397.

30. Cordon-Cardo C, Wartinger D, Petrylak D, Dalbagni G, Fair WR, Fuks Z, Reuter VE: Altered expression of the retinoblastoma gene product. Prognostic indicator in bladder cancer. J Natl Cancer Inst 1992; 84:1251-1256.

31. Otto T, Bex A, Schmidt U, Raz A, Rubben H: Improved prognosis assessment for patients with bladder carcinoma. Am J Pathol 1997; 150:1919-1923.

32. Lonn U, Lonn S, Friberg S, Nilsson B, Silfversward C, Stenkvist B (1995). Prognostic value of amplification of c-erb- B2 in bladder carcinoma. Clin Cancer Res 1: 1189-1194.

33. Bancroft JD, Marilyn Gamble (Ed), Theory and practice of histological techniques, Churchill Livingstone 2002.

34. McKenney JK, Amin MB. The role of immunohistochemistry in the diagnosis of urinary bladder neoplasms. Semin Diagn Pathol 2005;22(1):69-87.

35. Huang S, Minnassian H, More J D et al.Application of immunofluorescent staining in paraffin sections improved by trypsin digestion, Laboratory Investigation 35:383-391.

36. Miller K, Auld J, Jessup E, Rhodes A, Antigen unmarking by pressure cooker method. A comparison with microwave oven heating and traditional methods, Advances of anatomical pathology, 2:60-64.

37. Pluzek KY, Sweeney E,Miller KD, Isaacson P, A major advance for IHC Epos, J Pathol 169 (Suppl) abstract 220.

38. K Wester, P Ranefall et al. Automatic quantification of microvessel density in bladder carcinomas. British journal of cancer (1999) 81(8) 1363-1370.

39. Weidner (1995b) Intratumour microvessel density as a prognostic factor in cancer. Am J Pathol 147: 9-19

40. Zhau HE, Zhang X, von Eschenbach AC, et al. Amplification and expression of the c-erbB-2/neu proto-oncogene in human bladder cancer. Mol Carcinog. 1990;3:254–270.

41. Gorgoulis VG, Barbatis C, Poulias I, Karameris AM. Molecular and immunohistochemical evaluation of epidermal growth factor receptor and c-erb-B-2 gene product in transitional cell carcinomas of the urinary bladder: a study in Greek patients. Mod Pathol. 1995;8:758–764.

42. Moch H, Sauter G, Moore D, Mihatsch MJ, Gudat F, Waldman F. p53 and erbB-2 protein overexpression are associated with early invasion and metastasis in bladder cancer. Virchows Arch A Pathol Anat Histopathol. 1993;423:329–334.

43. Moriyama M, Akiyama T, Yamamoto T, et al. Expression of C-ERBB-2 gene product in urinary bladder cancer. J Urol. 1991;145:423–427.

44. Korkolopoulou P, Christodoulou P, Kapralos P, et al. The role of p53, MDM2 and c-erb B-2 oncoprotein, epidermal growth factor receptor and proliferation markers in the prognosis of urinary bladder cancer. Pathol Res Pract. 1997;93:767–775.

45. Sato K, Moriyama M, Mori S, et al. An immunohistologic evaluation of c erbB-2 gene product in patients with urinary bladder carcinoma. Cancer. 1992;70:2493–2498.

46. Tsigris C, Karayiannakis AJ, Zbar A, Syrigos KN, Baibas N, Diamantis T, et al. Clinical significance of serum and urinary c-erbB-2 levels in colorectal cancer. Cancer Lett 2002;184:215-22.

47. Tsigris C, Karayiannakis AJ, Syrigos KN, Zbar A, Diamantis T, Kalahanis N, et al. Clinical significance of soluble c-erbB-2 levels in the serum and urine of patients with gastric cancer. Anticancer Res 2002;22:3061-5.

48. Tambo M, Higashihara E, Terado Y, Nutahara K, Okegawa T. Comparison of serum HER2/neu with immunohistochemical HER2/neu expression for the prediction of biochemical progression in metastatic prostate cancer. Int J Urol 2009;16:369-74.

49. M. Lae, J. Couturier, S. Oudard, F. Radvanyi, P. Beuzeboc and A.Vieillefond. Assessing HER2 gene amplification as a potential target for therapy in invasive urothelial bladder cancer with a standardized methodology: results in 1005 patients. Annals of Oncology 21: 815–819, 2010.

50. Qihui J. Zhai, Jennifer Black, Alberto G. Ayala, and Jae Y. Ro (2007) Histologic Variants of Infiltrating Urothelial Carcinoma. Archives of Pathology & Laboratory Medicine: August 2007, Vol. 131, No. 8, pp. 1244-1256.

51. Lopez-Beltran, A ,J. Martin , J. Garcia , and M. Toro . Squamous and glandular differentiation in urothelial bladder carcinomas. Histopathology, histochemistry and immunohistochemical expression of carcinoembryonic antigen. Histol Histopathol 1988. 3:63–68.

52. Burry, A. F, S. R. Munn , E. P. Arnold , and C. U. McRae . Trophoblastic metaplasia in urothelial carcinoma of the bladder. Br J Urol 1986. 58:143–146.

53. Cardillo, M, V. E. Reuter , and O. Lin . Cytologic features of the nested variant of urothelial carcinoma: a study of seven cases. Cancer 2003. 99:23–27.

54. Drew, P. A, J. Furman , F. Civantos , and W. M. Murphy . The nested variant of transitional cell carcinoma: an aggressive neoplasm with innocuous histology. Mod Pathol 1996. 9:989–994.

55. Young, R. H. and L. R. Zukerberg . Microcystic transitional cell carcinomas of the urinary bladder: a report of four cases. Am J Clin Pathol 1991. 96:635–639.

56. Leroy, X, E. Leteurtre , A. De La Taille , D. Augusto , J. Biserte , and B. Gosselin . Microcystic transitional cell carcinoma: a report of 2 cases arising in the renal pelvis. Arch Pathol Lab Med 2002. 126:859–861.

57. Mai, K.T, G. Elmontaser , D. G. Perkins , H. M. Yazdi , W. A. Stinson , and A. Thijssen . Histopathological and immunohistochemical study of

papillary urothelial neoplasms of low malignant potential and grade associated with extensive invasive low-grade urothelial carcinoma. BJU Int 2004. 94:544–547.

58. Young, R. H. and E. Oliva . Transitional cell carcinomas of the urinary bladder that may be underdiagnosed: a report of four invasive cases exemplifying the homology between neoplastic and non-neoplastic transitional cell lesions. Am J Surg Pathol 1996. 20:1448–1454.

59. Amin, M. B, J. Y. Ro , and T. el-Sharkawy . et al. Micropapillary variant of transitional cell carcinoma of the urinary bladder: histologic pattern resembling ovarian papillary serous carcinoma. Am J Surg Pathol 1994. 18:1224–1232.

60. Johansson, S. L, G. Borghede , and S. Holmang . Micropapillary bladder carcinoma: a clinicopathological study of 20 cases. J Urol 1999. 161:1798–1802.

61. Lopez, J. I, K. Elorriaga , I. Imaz , and F. J. Bilbao . Micropapillary transitional cell carcinoma of the urinary bladder. Histopathology 1999. 34:561–562.

62. Aqoob, N, N. Kayani , J. Piryani , M. N. Sulaiman , and S. H. Hasan . Lymphoepithelioma-like carcinoma of urinary bladder: (LELCA). J Pak Med Assoc 2005. 55:402–403.

63. Zukerberg, L. R, N. L. Harris , and R. H. Young . Carcinomas of the urinary bladder simulating malignant lymphoma: a report of five cases. Am J Surg Pathol 1991. 15:569–576.

64. Sahin, A. A. , M. Myhre , J. Y. Ro , N. Sneige , R. H. Dekmezian , and A. G. Ayala . Plasmacytoid transitional cell carcinoma: report of a case

with initial presentation mimicking multiple myeloma. Acta Cytol 1991. 35:277–280.

65. Tamboli, P. , M. B. Amin , S. K. Mohsin , D. Ben-Dor , and A. Lopez-Beltran . Plasmacytoid variant of non-papillary urothelial carcinoma [abstract]. Mod Pathol 2000. 13:107A. 618.

66. Lee, H. I. , S. J. Jung , S. Lee , and J. Y. Ro . Plasmacytoid transitional cell carcinoma (P-TCC) of urinary bladder: a clinicopathologic study in 7 patients. Mod Pathol. 2005;18:151A. Abstract 698.

67. Eble, J. N. and R. H. Young . Carcinoma of the urinary bladder: a review of its diverse morphology. Semin Diagn Pathol 1997. 14:98–108.

68. Zukerberg, L. R, A. R. Armin , L. Pisharodi , and R. H. Young . Transitional cell carcinoma of the urinary bladder with osteoclast-type giant cells: a report of two cases and review of the literature. Histopathology 1990. 17:407–411.

69. Reuter, V. E. Sarcomatoid lesions of the urogenital tract. Semin Diagn Pathol 1993. 10:188–201.

70. Young, R. H. , M. R. Wick , and S. E. Mills . Sarcomatoid carcinoma of the urinary bladder: a clinicopathologic analysis of 12 cases and review of the literature. Am J Clin Pathol 1988. 90:653–661.

71. Eble, J. N. , G. Sauter , J. I. Epstein , and I. A. Sesterhenn . Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. Lyon, France: IARC Press; 2004. World Health Organization Classification of Tumours; vol 1.

72. Kotliar, S. N. , C. G. Wood , A. J. Schaeffer , and R. Oyasu . Transitional cell carcinoma exhibiting clear cell features: a differential diagnosis for clear cell adenocarcinoma of the urinary tract. Arch Pathol Lab Med 1995. 119:79–81.

73. Lopez-Beltran A, Luque RJ, Moreno A, Bollito E, Carmona E, Montironi R. The pagetoid variant of bladder urothelial carcinoma in situ A clinic pathological study of 11 cases. Virchows Arch 2002; 441:148–53.

74. Lopez-Beltran A, Cheng L, Andersson L, Brausi M, de Matteis A, Montironi R, et al. Preneoplastic non-papillary lesions and conditions of the urinary bladder: an update based on the Ancona International Consultation. Virchows Arch 2002;440:3–11.

75. Alvarez Kindelan J, Lopez Beltran A, Anglada Curado F, Moreno Arcas P, Carazo JL, Regueiro Lopez JC, et al. Clinico-pathologic differences between bladder neoplasm with low malignant potential and low-grade carcinoma. Actas Urol Esp 2001;25:645–50.

76. Antonio Lopez-Beltran,1, Rodolfo Montironi. Non-Invasive Urothelial Neoplasms: According to theMost RecentWHOClassification. European Urology 46 (2004) 170–176.

77. Holmang S, Andius P, Hedelin H, Wester K, Busch C, Johansson SL. Stage progression in Ta papillary urothelial tumors: relationship to grade, immunohistochemical expression of tumor markers, mitotic frequency and DNA ploidy. J Urol 2001;165:1124–8, discussion 1128–1130.

78. McKenney JK, Amin MB, Young RH. Urothelial (transitional cell) papilloma of the urinary bladder: a clinicopathologic study of 26 cases. Mod Pathol 2003;16:623–9.

79. Sauter G. Inverted papilloma. WHO Classification of Non-invasive Papillary Urothelial Tumors. In: Eble JN, Sauter G, Epstein JI, Sesterhenn I, editors. World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of the Urinary System and Male Genital Organs. Lyon: IARCC Press; 2004.

80. Dae sung cho et al. Prognostic Factors in Transitional Cell Carcinoma of the Upper Urinary Tract after Radical Nephroureterectomy. Korean J Urol. 2011 May; 52(5): 310–316.

81. Murphy WM (1999) Bladder cancer redefined. Cancer 86:1890–1892.

82. Bostwick DG, Mikuz G (2002) Urothelial papillary (exophytic) neoplasm. Virchows Arch 441:109–116.

83. Reuter VE, Melamed MR (1997) The urothelial tract. In: Stenberg SS (ed) Diagnostic surgical pathology. Lippincott Williams & Wilkins, Philadelphia.

84. Gregory T. MacLennan a, Ziya Kirkali b, Liang Cheng. Histologic Grading of Noninvasive Papillary Urothelial Neoplasms. european urology 51 (2007) 889–898.

85. J Hasan, R Byers and GC Jayson. Intra-tumoural microvessel density in human solid tumours. British Journal of Cancer (2002) 86, 1566 – 1577.

86. Coussens L, Yang-Feng TL, Liao YC, et al. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. Science. 1985;230:1132-1139.

87. Tanuja Rastogi et al. Cancer incidence rates among South Asians in four geographic regions: India, Singapore, UK and US. International Journal of Epidemiology 2008;37:147–160.

88. Mohammad Reza Jalali Nadoushan et al. Overexpression of HER-2/neu Oncogene and Transitional Cell Carcinoma of Bladder. Urologic oncology 2007 vol 4 no 3.

89. Surendra B. Kolla et al. Prognostic significance of Her2/neu overexpression in patients with muscle invasive urinary bladder cancer treated with radical cystectomy. Int Urol Nephrol (2008) 40:321–327.

90. J E martin et al. Clinical importance of squamous metaplasia in invasive transitional cell carcinoma of the bladder. J Clin Pathol 1989;42:250-253.

91. Matalka I et al. Transitional cell carcinoma of the urinary bladder: a clinicopathological study. Singapore Med J 2008; 49 (10):790.

92. Khaled El Gehani et al. Angiogenesis in urinary bladder carcinoma as defined by microvessel density (MVD) after immunohistochemical staining for Factor VIII and CD31. Libyan J Med 2011, 6: 6016.

93. William T. Stephenson et al. Analysis of Bladder Carcinoma by Subsite Cystoscopic Location May Have Prognostic Value. Cancer 66:1630-1635,1990.

94. Igor Frank et al. Transitional Cell Carcinoma of the Urinary Bladder with Regional Lymph Node Involvement Treated by Cystectomy-Clinicopathologic Features Associated with Outcome. 2003 American Cancer Society vol 97.

95. L. Santos et al. Neovascularisation is a prognostic factor of early recurrence in T1/G2 urothelial bladder tumours. Annals of Oncology 2003 14: 1419–1424.

96. Thomas Quentin et al. Alteration of the Vascular Endothelial Growth Factor and Angiopoietins-1 and -2 Pathways in Transitional Cell Carcinomas of the Urinary Bladder Associated with Tumor Progression. Anticancer research2004. 24: 2745-2756.

97. Ali Canoglu et al. Microvessel density as a prognostic marker in bladder carcinoma: Correlation with tumor grade, stage and prognosis. International Urology and Nephrology 2004**:** 401–405.

98. Eun Yong Choi et al. Microvessel density and its utility by CD 34 staining in bladder transitional cell carcinomas. Korean Journal of Urology 1999;40:1445-8.

99. Sari A et al. Evaluation of angiogenesis in urothelial carcinoma of bladder and its relation with prognostic factors. Ege Journal of Medicine / Ege Tip Dergisi 46(3) : 135 -140,2007.

100. E.A. Philp et al. Prognostic significance of angiogenesis in transitional cell carcinoma of the human urinary bladder. British Journal of Urology (1996), 77. 352–357.

101. N. E. Stavropoulos et al. Prognostic significance of angiogenesis in superficial bladder cancer. International Urology and Nephrology 2004;36: 163–167.

102. Donna E. Hansel et al. HER2 Overexpression and Amplification in Urothelial Carcinoma of the Bladder Is Associated With MYC

Coamplification in a Subset of Cases. Am J Clin Pathol 2008;130:274-281.

103. Aurora Alexa et al. Clinical significance of Her2/neu overexpression in urothelial carcinomas. Romanian Journal of Morphology and Embryology 2010, 51(2):277–282.

104. Rawaa G Al-Tereihi et al. Overexpression of HER-2\neu receptor protein in Urinary Bladder Carcinoma, An Immunohistochemical Study. Fac Med Baghdad 2011; Vol. 53, No. 2.

105. C.K. Hawke et al. Microvessel density as a prognostic marker for transitional cell carcinoma of the bladder. British Journal of Urology (1998), 81, 585–590.

106. Jonathan C. Goddard et al. Microvessel Density at Presentation Predicts Subsequent Muscle Invasion in Superficial Bladder Cancer. Clin Cancer Res 2003;9:2583-2586.

107. Z. Ahmed,S et al. Transitional cell carcinomas of urinary bladder. Ahistopathological study. Journal of Pakistan medical association. September, 2002.

108. Zaheer Alam et al. Predictors of Lymph node involvement in bladder cancer treated with radical cystectomy. Journal of Pakistan medical association. August 2009.

ANNEXURE I

WHO CLASSIFICATION OF BLADDER CARCINOMAS

UROTHELIAL TUMOURS

Infiltrating urothelial carcinoma

- with squamous differentiation
- with glandular differentiation
- with trophoblastic differentiation
- Nested
- Microcystic
- Micropapillary
- Lymphoepithelioma-like 3
- Lymphoma-like
- Plasmacytoid
- Sarcomatoid
- Giant cell
- Undifferentiated

Non-invasive urothelial neoplasias

- Urothelial carcinoma in situ
- Non-invasive papillary urothelial carcinoma, high grade
- Non-invasive papillary urothelial carcinoma, low grade
- Non-invasive papillary urothelial neoplasm of low malignant potential
- Urothelial papilloma
- Inverted urothelial papilloma

SQUAMOUS NEOPLASMS

Squamous cell carcinoma

Verrucous carcinoma

Squamous cell papilloma

GLANDULAR NEOPLASMS

Adenocarcinoma

- Enteric
- Mucinous
- Signet-ring cell
- Clear cell

Villous adenoma

NEUROENDOCRINE TUMOURS

Small cell carcinoma

Carcinoid

Paraganglioma

MELANOCYTIC TUMOURS

Malignant melanoma

Nevus

MESENCHYMAL TUMOURS

Rhabdomyosarcoma

Leiomyosarcoma

Angiosarcoma

Osteosarcoma

Malignant fibrous histiocytoma

Leiomyoma

Haemangioma

Others

HAEMATOPOIETIC AND LYMPHOID TUMOURS

Lymphoma

Plasmacytoma

MISCELLANEOUS TUMOURS

Carcinoma of Skene, Cowper and Littre glands

Metastatic tumours and tumours extending from other organs

ANNEXURE II

TNM STAGING OF BLADDER CARCINOMAS

T– Primary tumour

TX Primary tumour cannot be assessed

T0 No evidence of primary tumour

Ta Non-invasive papillary carcinoma

Tis Carcinoma in situ: "flat tumour"

T1 Tumour invades subepithelial connective tissue

T2 Tumour invades muscle

T2a Tumour invades superficial muscle (inner half)

T2b Tumour invades deep muscle (outer half)

T3 Tumour invades perivesical tissue:

T3a Microscopically

T3b Macroscopically (extravesical mass)

T4 Tumour invades any of the following: prostate, uterus, vagina, pelvic wall or abdominal wall

T4a Tumour invades prostate, uterus or vagina

T4b Tumour invades pelvic wall or abdominal wall

N – Regional lymph nodes

NX Regional lymph nodes cannot be assessed

N0 No regional lymph node metastasis

N1 Metastasis in a single lymph node 2 cm or less in greatest dimension

N2 Metastasis in a single lymph node more than 2 cm but not more than 5 cm in greatest dimension, or multiple lymph nodes, none more than 5 cm in greatest dimension

N3 Metastasis in a lymph node more than 5 cm in greatest dimension

M – Distant metastasis

MX Distant metastasis cannot be assessed

M0 No distant metastasis

M1 Distant metastasis

STAGE GROUPING

Stage 0a	Ta	N0	M0
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2a, b	N0	M0
Stage III	T3a, b	N0	M0
	T4a	N0	M0
Stage IV	T4b	N0	M0
	Any T	N1, N2, N3	M0
	Any T	Any N	M1

ANNEXURE III

IMMUNOHISTOCHEMISTRY PROCEDURE

1. 4µ thick sections were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chrome alum coated slides.
2. The slides were incubated at 58ºC for overnight.
3. The sections were deparaffinized in xylene for 15 minutes x 2 changes.
4. The sections were dehydrated with absolute alcohol for 5 minutes x 2 changes.
5. The sections were washed in tap water for 10 minutes.
6. The slides were then immersed in distilled water for 5 minutes.
7. Heat induced antigen retrieval was done with microwave oven in appropriate temperature with appropriate buffer for 20 to 25 minutes.
8. The slides were then cooled to room temperature and washed in running tap water for 5 minutes.
9. The slides were then rinsed in distilled water for 5 minutes.
10. Wash with appropriate wash buffer (citrate buffer) for 5 minutes x 2 changes.
11. Apply peroxidase block over the sections for 10 minutes.
12. Wash the slides in citrate buffer for 5 minutes x 2 changes.
13. Cover the sections with power block for 15 minutes.
14. The sections were drained (without washing) and appropriate primary antibody was applied over the sections and incubated for 45 minutes.
15. The slides were washed in citrate buffer for 5 minutes x 2 changes.
16. The slides were covered with SuperEnhancer for 30 minutes.
17. The slides were washed in citrate buffer for 5 minutes x 2 changes.
18. The slides were covered with SS Label for 30 minutes.
19. Wash in citrate buffer for 5 minutes x 2 changes.

20. DAB substrate was prepared by diluting 1 drop of DAB chromogen to 1 ml of DAB buffer.
21. DAB substrate solution was applied on the sections for 8 minutes.
22. Wash with citrate buffer solution for 5 minutes x 2 changes.
23. The slides are washed well in running tap water for 5 minutes.
24. The sections were counterstained with Hematoxylin stain for 2 seconds (1 dip).
25. The slides are washed in running tap water for 3 minutes.
26. The slides are air dried, cleared with xylene and mounted with DPX.

ABSTRACT

AIM:

The variable prognosis of bladder cancer within a pathological stage necessitates the identification of subgroups of patients with a more aggressive disease. The role of microvessel density and HER-2/neu expression in transitional cell carcinomas of bladder is far from being fully established. The aim of the present study was to identify the incidence and distribution of bladder carcinoma in patients admitted in the Rajiv Gandhi Government General Hospital, Chennai in the year 2010 and 2011 and to evaluate the role of microvessel density and HER-2/neu expression in transitional cell carcinomas of bladder and correlate the findings with several clinico-pathological features and prognosis.

MATERIALS AND METHODS:

Formalin-fixed paraffin-embedded tissue samples from 50 patients of transitional cell carcinoma of bladder in the year 2010 to 2011 were studied by immunohistochemistry, using monoclonal antibodies to CD 34 and HER-2/neu. Microvessel density was found from CD 34 expression. The results were correlated with clinico-pathological features and prognostic factors.

RESULTS:

Microvessel density was significantly related with male gender, non-papillary tumours and with necrosis. Higher HER-2/neu expression correlated significantly with grade and urine cytology positivity.Increasing HER-2/neu expression correlated with age, male gender, multiple tumours, tumours with increased size, stage, infiltration, necrosis, sarcomatoid component, necrosis and with recurrence. Increasing

microvessel density was associated with multiple tumours, higher grade, tumours with sarcomatoid component, positive urine cytology and in recurrence. Though microvessel density seemed to be decreasing with stage, the patients with high microvessel density (>50 vessels/200X) showed an increased rate of recurrence.

CONCLUSION:

This study showed that increasing values of microvessel density and HER-2/neu expression were found in patients with increasing stage, grade and recurrence. Thus they can be good prognostic factors for assessing patients presenting with early stage for the first time. A larger sample size will help in identifying the association between the stage, recurrence, microvessel density status and HER-2/neu expression.